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GENETIC CONTROL OF ARYLESTERASE IN THE PIG

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I. INTRODUCTION

BLOOD plasma of pigs contains two esterases, a butyrylcholinesterase and an acetylarylesterase (AUGUSTINSSON, 1958, 1959; AUGUSTINSSON and OLSSON, 1959 a). Both enzymes hydrolyse aromatic esters (*e.g.*, phenyl acetate), arylesterase being approximately 100 times more active toward this substrate than cholinesterase when hydrolysis rates are compared on a nitrogen content basis, using purified enzymes. In contrast to the activity of cholinesterase, the arylesterase activity exhibits considerable individual variation. The activity values for arylesterase obtained with pig plasma and phenyl acetate range from 0—200, when activity determinations are carried out in the presence of prostigmine, which selectively inhibits cholinesterase.

When studying the transference of esterases from the sow to the offspring (AUGUSTINSSON and OLSSON, 1959 b) it appeared that plasma from new-born piglets had no or very low activity in hydrolysing phenyl acetate, in spite of a high activity in the plasma of their mothers. Consequently, there is no transmission of arylesterase from mother to offspring in utero, neither is possible an absorption from colostrum and milk, since both lack this enzyme. This low enzyme activity in the plasma of new-born piglets and also of certain adult animals is almost entirely due to cholinesterase. Previous experiments also demonstrated that the increase in plasma arylesterase activity of some piglets in the litter during the first weeks of life runs parallel with the increase in plasma albumin concentration. There is no esterase inhibitor present in plasma of low esterase activity. Evidence has not yet been conclusively presented to show which organ (*e.g.*, liver) is the main site of

biosynthesis of this enzyme (AUGUSTINSSON, GRANT, OLSSON and THAFVELIN, 1960).

The results of our first experiments, carried out in the beginning of 1958 (AUGUSTINSSON and OLSSON, 1959 b), gave rise to the idea that arylesterase is genetically determined. More recent studies with backcrosses and a great number of cross tests with pigs of various phenotypes (*i.e.*, animals with various plasma arylesterase activity levels), have confirmed this proposal and made it possible to put forward the following hypothesis for gene interaction in the formation of plasma arylesterase in the pig (AUGUSTINSSON and OLSSON, 1960).

The various phenotypes of adult animals are produced by a set of multiple alleles:

Allele	Activity Level
a	0
A_1	25
A_2	50
A_3	75
A_4	100

Each allele determines a certain plasma arylesterase activity, and the distinguishable phenotypes among animals (at least two month of age) are the manifestation of additive effects of two alleles, *e.g.*,

Genotype	Phenotype
aa	0
aA_2	50
A_1A_1	50
A_1A_3	100
A_2A_3	125

This hypothesis is strongly supported by the experimental results obtained, as will be presented in the following report, which is based on all tested litters and all mating experiments performed from February 1958 to September 1960.

II. MATERIAL AND METHODS

Animals. The genetic analyses were carried out with animals of Swedish Landrace ("Svensk lantras") and Yorkshire breeds. Particularly extensive experimental matings were performed with boar "245" and his offspring (Fig. 2 and Table 2). Parents and offspring to animals

TABLE 1. *Mating scheme.*

Numbers refer to mating No. in Table 2. L and Y designate Swedish Landrace and Yorkshire breeds, respectively. Numbers in italics refer to back-crosses and F_2 tests. For mating Nos. 40 and 50, not referred to in this table, see legends to Table 2. Sows grouped together in brackets are sisters.

Sows	Boars														
	245 LL	38 LL	380 LL	426 LL	295[1] LL	1314[2] LL	4433 LL	1233 YY	260 LL	1070 YY	1274 YY	430 YY	1247 LL	134 LL	565 YY
33 LL	1	—	—	—	—	—	—	—	—	—	—	—	61	66	
27 LL	—	—	—	—	—	—	—	—	—	—	—	—	62		
940 LL	*2*	—	—	—	—	—	—	—	—	—	—	—	—	67	
936 LL	*9, 10*	—	—	—	—	34									
966 LL[3]	*11*														
967 LL[3]	*3, 4*	—	—	24											
963 LL[3]	*5*														
974 LL[4]	Fig. 2	—	—	—	32										
413 LL	14, 15	—	—	—	33	—	—	—	—	—	—	—	*63, 64*		
418 LL	—	—	—	—	—	—	—	—	—	—	—	—	*65*		
1119 LL	—	—	—	—	—	—	—	—	—	—	—	—	—	*68*	
162 LL	6, 7														
145 LL	8														
7 LL	12, 13														
297 LL	—	—	—	—	*31*										
1315 LL	—	—	—	—	—	*35*									
1316 LL	—	—	—	—	—	*36*									
269 LL	—	—	—	—	—	—	41								
343 LL	—	—	22												
222 YL	—	—	—	—	—	—	—	—	—	—	—	—	—	—	70
225 YL	—	—	—	—	—	—	—	—	—	—	57				
70 YY	—	—	—	—	—	—	—	—	—	—	56				
94 LY	—	—	20	—	—	—	38								
14 LL	—	—	21	—	—	—	—	—	46						
115 YL	—	—	—	—	—	—	—	—	—	54					
31 LL	—	16	—	28	—	—	37								
54 LY	—	—	—	30	—	—	40								
55 LY	—	18	—	29	—	—	39								
95 YY	—	—	—	—	—	—	—	—	—	—	—	—	—	—	69
102 YY	—	—	—	—	—	—	—	—	—	53	58				
153 LY	—	—	23	—	—	—	—	—	47						
19 LL	—	—	—	—	—	—	—	—	43						
21 LL	—	17	—	—	—	—	—	—	45						
314 LY	—	—	—	26											
132 LL	—	—	—	25											
122 YL	—	—	—	—	—	—	—	—	—	—	—	59			
127 YL	—	—	—	—	—	—	—	42	—	52					
350 YL[5]	—	—	—	—	—	—	—	—	—	—	—	60			
356 YL[5]	—	—	—	—	—	—	—	—	—	51	55				
577 YY	—	19	—	—	—	—	—	—	48						
1 YL[6]	—	—	—	27											
8 YL[6]	—	—	—	—	—	—	—	—	44						

[1] Out of ♀ 963 × ♂ 245.
[2] Out of ♀ 413 × ♂ 245.
[3] Out of ♀ 7 × ♂ 245.
[4] Out of ♀ 162 × ♂ 245.
[5] Out of ♀ 577.
[6] Out of ♀ 577 × ♂ 38.

from the extensive series of cross breeding experiments between the two breeds at the Animal Breeding Institute, Wiad (Sweden) were also tested. All types of mating investigated are tabulated in Table 1.

Blood plasma. Blood samples were collected with heparin from sows and boars without anaesthesia by puncture of an ear vein and from piglets by puncture of the anterior vena cava. The plasma was centrifuged free from all cells including thrombocytes. In some instances the plasma was preserved by freezing, which resulted in the formation of fibrin clots; these were removed by centrifuging (or by hand) before using the plasma. In some cases, the plasma was difficult to obtain free from some signs of haemolysis, but this did not influence the results, as was shown by control experiments. In most experiments, blood samples were collected when the animals were 50 to 70 days old, when the arylesterase activity had reached adult values.

In studies on the variation of esterase activity during the course of lactation and suckling, blood samples were collected from the sows 2—4 days before expected parturition, at parturition and 36 hours and 3 days after parturition. The piglets were removed from the sow immediately after birth and were not allowed to suckle. Blood samples from the cords (after cutting) of each piglet were pooled. All piglets of the litter were then allowed to suckle and subsequent blood samples from individual piglets were collected at the age of 36 hours, 3 days and then weekly until the age of about 56 days. It will be noted that all piglets of the litter lived together under the same surrounding conditions.

Assay of arylesterase activity. The esterase activity was measured by the Warburg technique at 25° C in a bicarbonate-CO_2 buffer (33.6 mM $NaHCO_3$ and 1.0 mM $CaCl_2$, pH 7.4) and was expressed in μl CO_2 evolved per 0.02 ml plasma per 30 min.* Corrections were made for spontaneous hydrolysis of substrate and for hydrolysis catalysed by cholinesterase, by carrying out the determinations in the presence of prostigmine which selectively inhibits cholinesterase activity. Phenyl acetate in a final concentration of 10 mM was used as substrate throughout the experiments.

* In previous reports (AUGUSTINSSON and OLSSON, 1959 a, b) the values for arylesterase activity were expressed per 0.1 ml plasma, and were not corrected for cholinesterase activity. These values, therefore, are not directly comparable with those given above; they may be recalculated, however, by dividing previous values with 4 and subtracting 10, which is the mean activity value per 0.02 ml plasma for cholinesterase hydrolysing phenyl acetate.

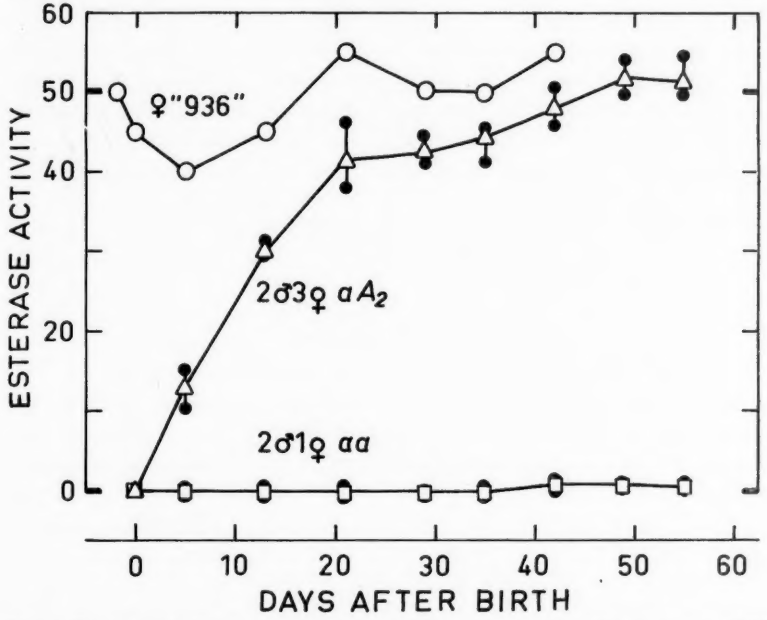


Fig. 1. Plasma arylesterase activity of sow "936", back-crossed with her father "245" (mating No. 9 in Table 2), and their eight piglets during the course of lactation. Last values for the sow also indicates weaning. Esterase activity in μl CO_2. ○, sow "936"; △ and □, mean activity values; ●, highest and lowest values of piglet plasmata. Esterase activity of piglet plasmata at birth was measured with a pooled sample collected from the cords.

III. RESULTS

1. Plasma arylesterase activity of piglets during the suckling period

It was demonstrated in our first experiments (AUGUSTINSSON and OLSSON, 1959 b) that the plasma of all new-born animals had no or very low arylesterase activity, irrespective of high or medium activity in the plasma of their parents. Differentiation into groups began on the second to fourth day of life, and the activity reached adult values at the age of about 50 days. Since then, this observation has been confirmed in a number of experiments; the results obtained in a back-cross test are illustrated in Fig. 1 (*cf.* also Fig. 2).

A daughter ("936") of phenotype 50 (genotype aA_2) was crossed with

her father (genotype *aa*) (mating No. 9 in Table 2). A pooled sample from the cords of the eight piglets showed no arylesterase activity. During the suckling period the plasma activity of five piglets in the litter increased gradually, and reached the same activity (50) as that of their mother at the end of this period. There was no activity in the plasma of the remaining three piglets when these had reached maturity. From the results of a number of similar experiments (see Table 2), the conclusion was drawn that the property of pig plasma to hydrolyse phenyl acetate (as a measure of arylesterase activity) is inherited.

2. Genetic analyses

A. *Segregation in back-crosses and F_2 generations*

In our first experiments (AUGUSTINSSON and OLSSON, 1959 b) the phenotypes of the offspring could be divided into four groups, corresponding to the esterase activities 75, 50, 25 and 0. One sow from each group of the offspring from a boar ("245") of phenotype 0 (zero) was used in back-cross tests, summarized in Fig. 2. A litter from a sow of phenotype 100 ("7") segregated into two phenotypes, 75 and 25. In back-crosses the offspring from sows of either of these phenotypes again segregated into two groups, 75 and 0 (out of "966") and 25 and 0 (out of "963" and "967", respectively). It was concluded from these results and from matings with sows of phenotype 0 (Nos. 1 and 2 in Table 2) that the father was homozygous for the gene *a*, responsible for 0 (zero) activity and that two alleles, A_3 and A_1, determined the activity of phenotypes 75 and 25, respectively, which consequently corresponded to the heterozygous genotypes aA_3 and aA_1. The mother ("7") of this offspring was obviously of genotype A_1A_3. Similar results were obtained with the sows of phenotype 50 ("162" and "25"); the phenotype of sow "25" was not determined before slaughter but she was by inference also of phenotype 50. From the result of back-cross tests it was concluded that the genotype of these sows was aA_2.

From analyses of several litters and their parents it was clear that at least four phenotypes exist. A phenotype 100 was already observed with sow "7" (see above), the genotype of which was inferred to be A_1A_3. Therefore, genotypes A_3A_3 and also A_2A_3 should exist. Actually all possible combinations of the four alleles, *a*, A_1, A_2 and A_3, have been observed. When activities were higher than 100 it was in some cases difficult (for technical reasons, sensitivity of the assay method, etc.) to differentiate between adjacent phenotypes, especially between pheno-

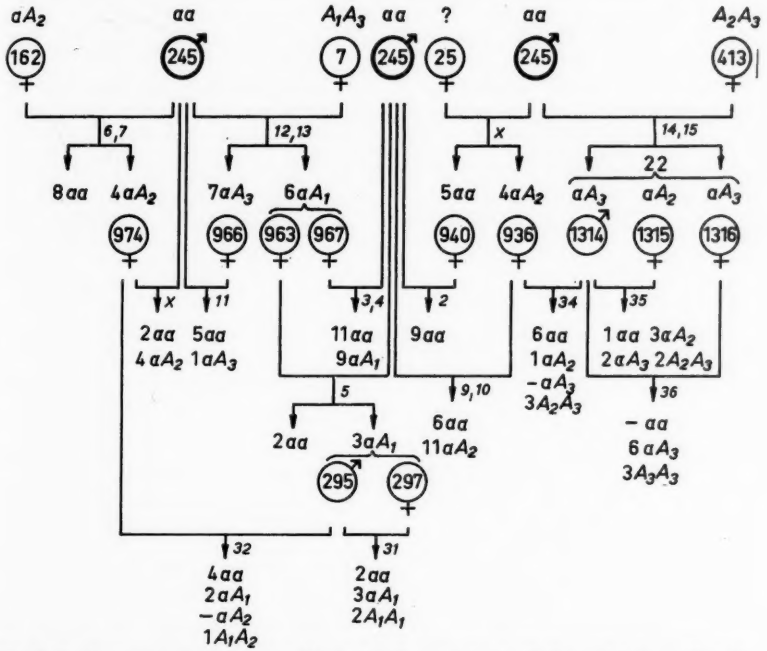


Fig. 2. Back-crosses and F_2-generations. Small numbers in italics refer to mating No. in Table 2; X denotes mating studied but not shown in Table 2. The distribution of phenotypes in the progeny of all back-crosses with boar "245", in which the segregation was expected to be 1:1, constitutes good statistical agreement ($\chi^2=0.074$, $0.80>P>0.70$).

types 100 and 125 and between 125 and 150. Moreover, as will be discussed more fully below, the activity of mature boars was in several cases lower than could be surmised from the mating experiments. However, it was concluded from back-cross tests and F_2-tests that boar "1247" was of the genotype A_2A_2. In back-crosses with two daughters of this boar, sow "413" and "418", it was found that the offspring belonged to the phenotypes 125 and 150, corresponding to the genotypes A_2A_2 and A_2A_3 (Nos. 63—65, Table 2). The genotype A_2A_3 for sow "413" was proved by the phenotypic ratios obtained in the F_2-generation from crosses with boar "245" (genotype aa). Thus, in two cross tests all 22 piglets (Nos. 14 and 15) were either of the phenotype 50 or 75 (genotypes aA_2 and aA_3). In crossing a boar ("1314") from one of these litters with two of his sisters ("1315" and "1316") it was established that

"1314" was of genotype aA_3 and also that his two sisters were of genotype aA_2 and aA_3, respectively. Further proof for the genotype aA_3 of boar "1314" was obtained in a cross test with a sow of genotype aA_2 ("936") who had the same father ("245") as boar "1314" and the genotype of which had been established in back-cross tests.

Still another back-cross test was performed with boar "134" and his daughter "1119", the phenotype of which was 75 corresponding to genotype aA_3 (mating No. 68 in Table 2); her mother (not further studied in this series of experiments) was found to have zero arylesterase activity (genotype *aa*). In this back-cross test the offspring was of phenotypes 75 and 150, and we therefore concluded that their father was of the homozygous genotype A_3A_3. This was established in crossing this boar with two sows of phenotype 0 when the offspring was of one single heterozygous genotype aA_3 in both cases (Nos. 66 and 67).

B. *Further types of mating studied*

In order to obtain further evidence for the hypothesis that the various phenotypes of pigs are produced by the additive effect of two alleles, each determining an arylesterase activity of 25 or a multiple of 25, the progeny of a great variety of types of mating was studied. The following matings are particularly notable, references being made to mating No. in Table 2, which, with few exceptions (found in Fig. 2), summarizes all matings studied.

In all matings (*e.g.*, Nos. 1 and 2) where both parents were of phenotype 0, the offspring was always of this same phenotype. Consequently, all these animals must be homozygous for gene *a*. When a boar of this genotype was crossed with sows of phenotype 25 (Nos. 3—5), the offspring were of two phenotypes, 0 and 25. It was easily concluded that the mother and her offspring of activity 25 were of genotype aA_1. Similar segregation into two groups was found for the offspring from a boar of the homozygous genotype *aa* and sows of phenotypes 50 and 75,

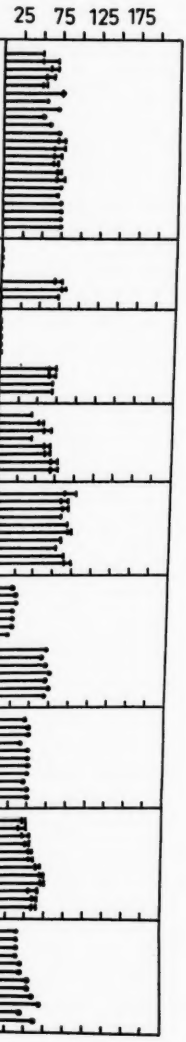
All matings are arranged with increasing plasma arylesterase activity of boars, sows and offspring. Mating No. is that found in Table 1; numbers in italics designate back-cross and F_2-tests. Both the boar and the two sows in matings Nos. 49 and 50, not listed in Table 1, were of Swedish Landrace. The phenotypic values in the histogram refer to arylesterase activities in μl CO_2 per 30 min. For each phenotype the activity values obtained with male piglets are shown first, followed by the values obtained with female piglets. Highest and lowest activities obtained with two or more samples taken from the same individual at different occasions (when the animals were 50 to 75 days old) are designated by ||; a dot (·) designates the activity value obtained with one single sample analysed.

TABLE 2. *Plasma arylesterase activity of the progeny of various types of mating.*

Mating No.	PARENTS genotype – phenotype ♂	PARENTS genotype – phenotype ♀	OFFSPRING number ♂	OFFSPRING number ♀	OFFSPRING genotype	OFFSPRING phenotype 25 75 125 175
1		aa 0	5	3	aa	
2		aa 0	4	5	aa	
3,4		aA_1 25	2	9	aa	
			4	5	aA_1	
5		aA_1 25	–	2	aa	
			2	1	aA_1	
6,7	aa 0	aA_2 50	4	4	aa	
			1	3	aA_2	
8		aA_2 50	1	3	aa	
			3	1	aA_2	
9,10		aA_2 50	4	2	aa	
			5	6	aA_2	
11		aA_3 75	4	1	aa	
			1	–	aA_3	
12,13		A_1A_3 100	–	6	aA_1	
			3	4	aA_3	

TABLE 2. Continued.

14,15	$\alpha\alpha$ 0	A_2A_3 150-175	10 12	αA_2, αA_3
16	$\alpha\alpha$ 0	αA_3 75	2 1	$\alpha\alpha$
			1 2	αA_3
17		αA_3 75	3 2	$\alpha\alpha$
			1 3	αA_3
18		A_2A_3 125-150	3 5	αA_2, αA_3
19		A_3A_3 150-175	3 7	αA_3
20	$\alpha\alpha$ 0	A_1A_3 100	3 4	αA_1
			4 3	αA_3
21		A_2A_2 100	4 7	αA_2
22		A_2A_3 125	9 3	αA_2, αA_3
23		A_2A_3 125	10 2	αA_2, αA_3



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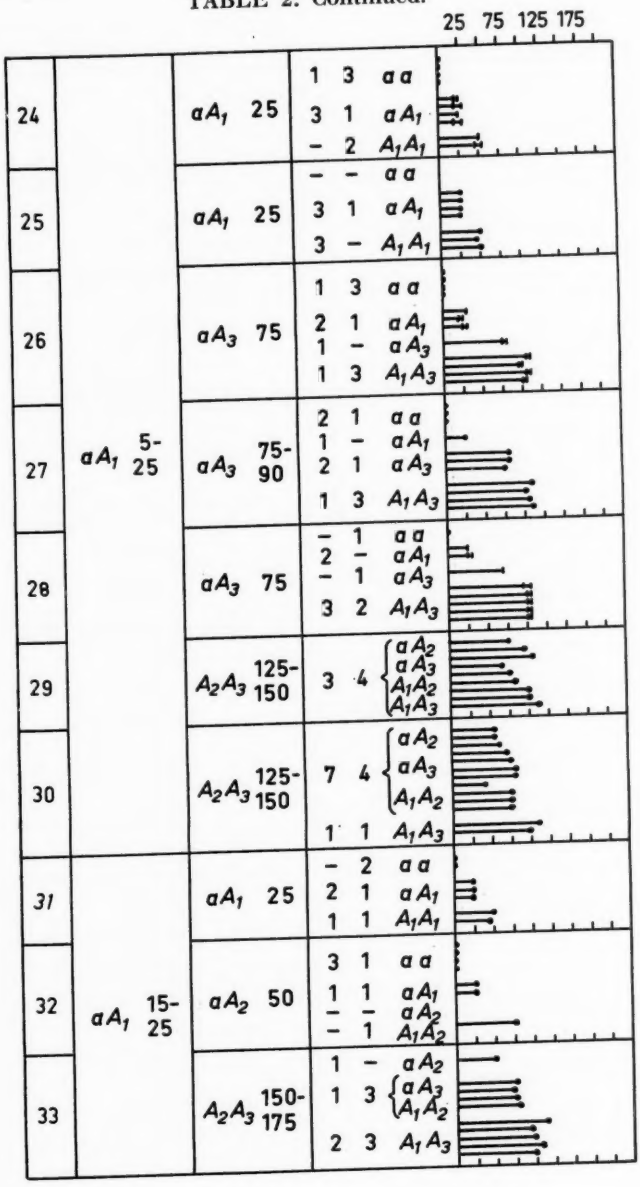

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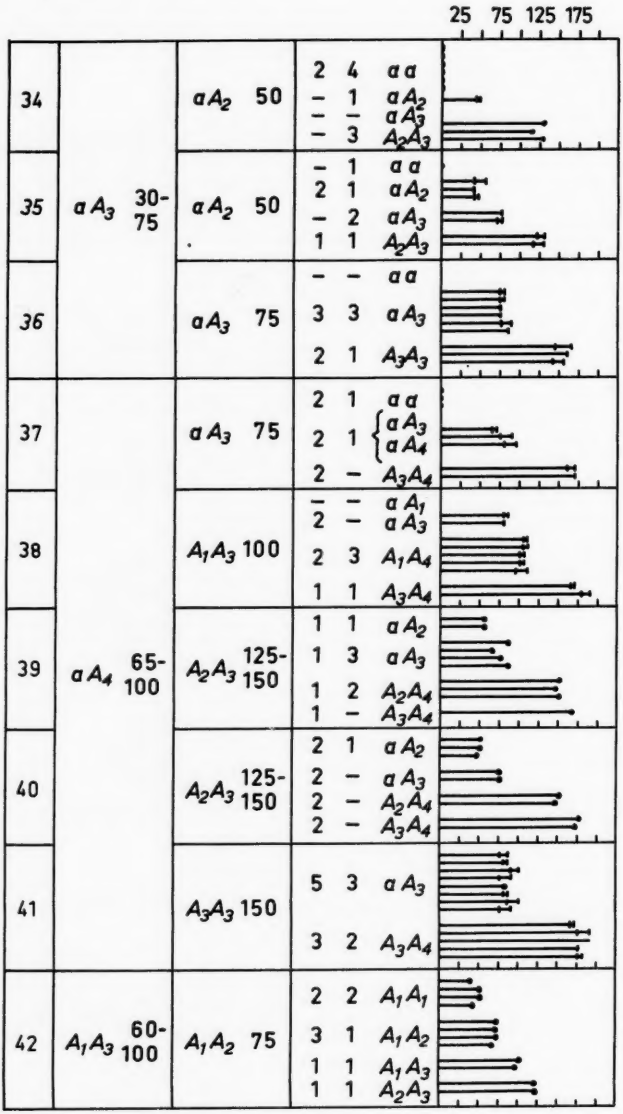

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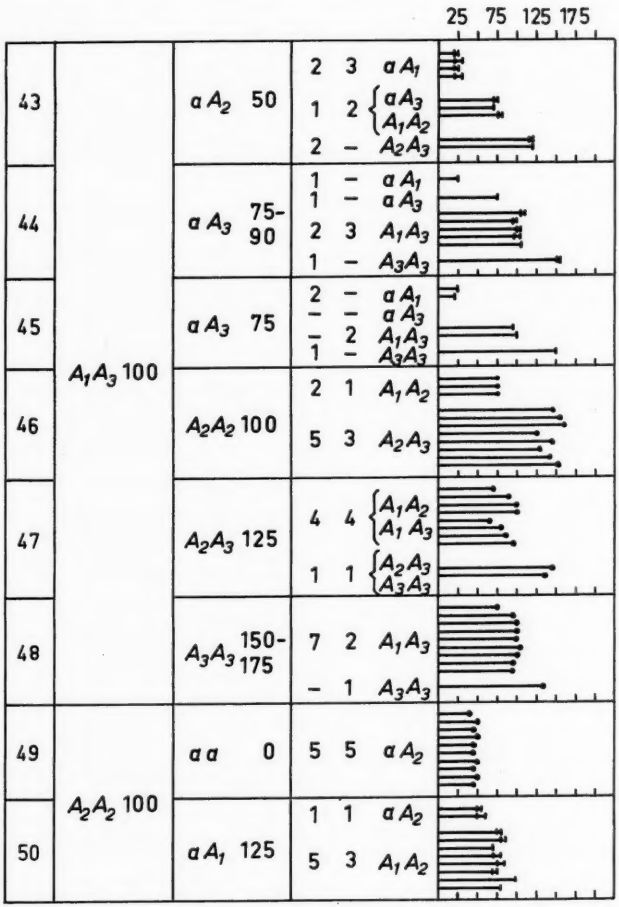

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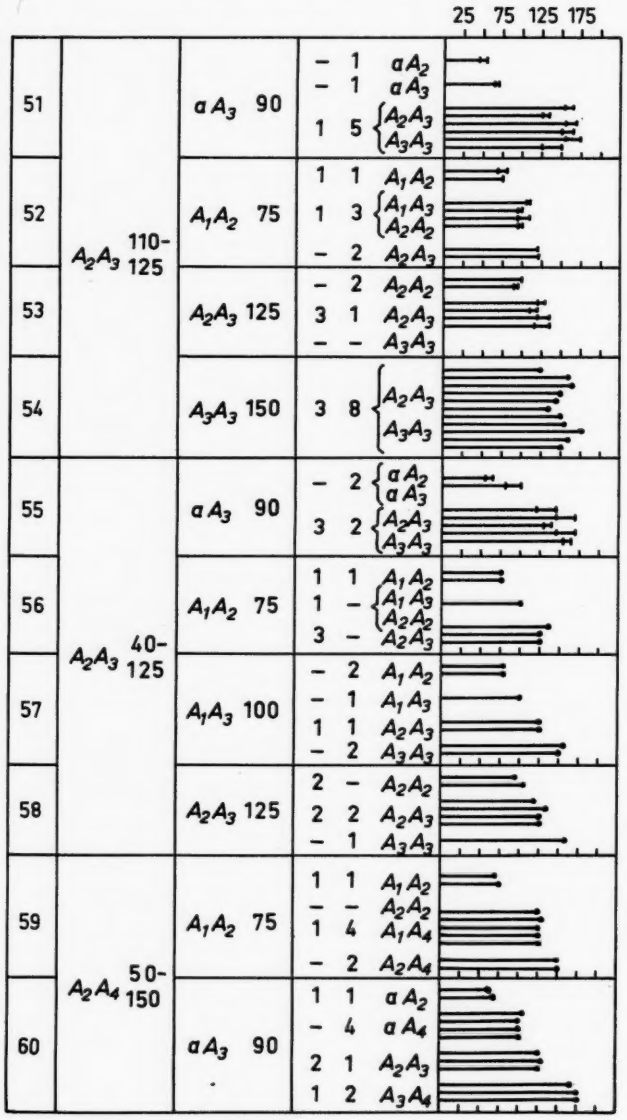

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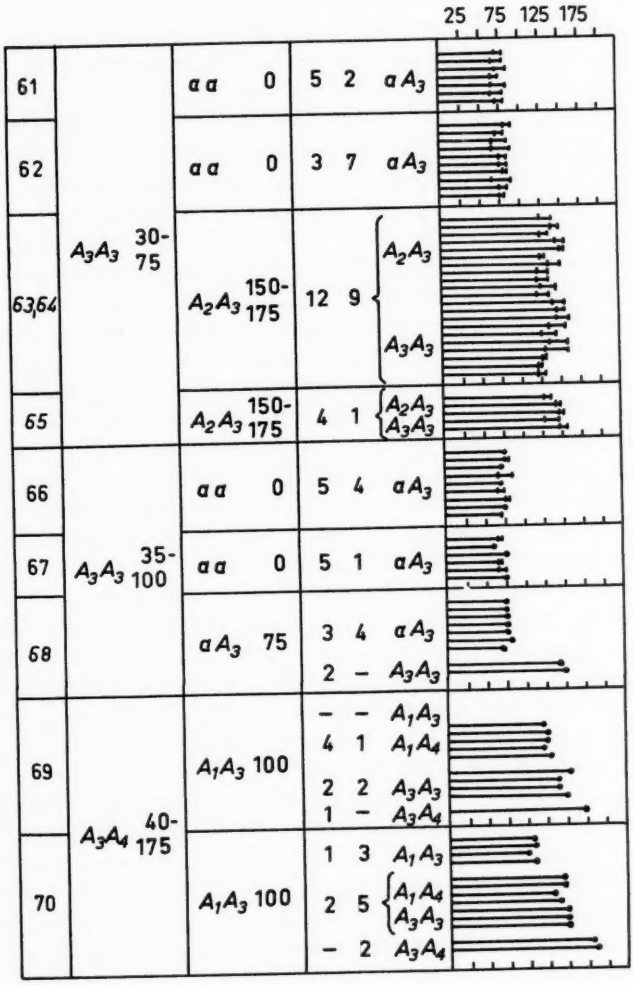

respectively, provided these were of a heterozygous genotype, aA_3 (Nos. 6—10) and aA_3 (Nos. 11 and 16), respectively.

In matings with a boar of this same genotype *aa* and sows of phenotype 100, the offspring was either of one phenotype (50) or two phenotypes (75 and 25). This means that the sow in the first mating (No. 21) was homozygous for gene A_2 and in the second one (No. 20) heterozygous for the genes A_1 and A_3. One single phenotype of the offspring was obtained, as expected, when a sow of homozygous genotype *aa* was crossed with boars of homozygous genotypes, as A_2A_2 (No. 49) and A_3A_3 (Nos. 61, 62, 66, 67).

In matings where both parents were of heterozygous genotypes, there will be theoretically three or four groups of phenotypes in the offspring. Therefore, the probability of a missing phenotypic group in a litter is fairly great, and actually this was the experimental result in some cases (Nos. 25, 32, 34, 36, 38, 45, 53, 59, 69). When both parents had the *same* plasma arylesterase activity (phenotype 25), three phenotypes (0, 25 and 50) were obtained in the offspring, corresponding to the three kinds of genotype, *aa*, aA_1 and A_1A_1. This is exemplified by mating Nos. 24 and 31. These and other matings (Nos. 26—28, 34, 35, 37) in which it was demonstrated that both parents were heterozygous and carrier of gene *a*, are examples where the offspring of parents both having arylesterase activity segregates into a group with no activity (phenotype 0). Parents of phenotype 125, the genotype of which was by inference A_2A_3, gave an offspring of three phenotypes, 100, 125 and 150, corresponding to the three genotypes A_2A_2, A_2A_3 and A_3A_3 (No. 58).

The offspring was also segregated into three phenotypes in some matings between parents of different heterozygous genotypes. In mating No. 33, for instance, the boar had genotype aA_1 and the sow A_2A_3. One of the three phenotypes obtained (75) could be composed of two genotypes, aA_3 and A_1A_2. Similar results were obtained in other cross tests, *e.g.*, Nos. 29 and 30. Other notable results are those obtained in crossing ♀ $aA_2 \times$ ♂ A_1A_3 (No. 43) where one of the three phenotypes in the offspring also could correspond to either genotype aA_3 or A_1A_2, and in the cross tests ♀ $A_1A_2 \times$ ♂ A_2A_3 (Nos. 52 and 56), where the phenotype 100 of the offspring corresponded to genotypes A_1A_3 or A_2A_2.

The existence of a set of multiple alleles is also supported by the observation of four phenotypes in the progeny of crossing a boar of genotype aA_1 with sows of various heterozygous genotypes. The progeny in matings with sows of phenotype 75 (genotype aA_3) gave the four expected phenotypes in the progeny (Nos. 26—28). Four expected

groups were also obtained in the offspring from matings ♀ $aA_2 \times$ ♂ aA_3 (No. 35; in No. 34, one phenotype missing), ♀ $aA_3 \times$ ♂ A_1A_3 (No. 44; *cf.* No. 45), ♀ $A_1A_2 \times$ ♂ A_1A_3 (No. 42), and ♀ $A_1A_3 \times$ ♂ A_2A_3 (No. 57).

In the series of matings studied we have also observed animals carrying a gene A_4. By a series of cross tests (Nos. 37—41) with a boar of phenotype 100 and sows of known genotypes, it was concluded that the genotype of the boar was aA_4. In the offspring of this boar we found, among others, genotypes A_1A_4, A_2A_4 and A_3A_4. Another boar of phenotype 150 could be classified as belonging to a genotype A_2A_4; particularly notable is the result of mating with a sow of genotype aA_3 (No. 60) in which all four expected phenotypes were found in the litter. Finally, the genotype of a boar of phenotype 175 was shown to be A_3A_4 (Nos. 69 and 70).

3. Esterase activity in relation to sex and breed

There are no sex differences between arylesterase activity of pig plasma, as is obvious from the results reported in Fig. 1 and Table 2. The activity, however, is probably influenced by sex hormones, as will be briefly discussed below.

No differences in the genetic system for the synthesis of plasma arylesterase have been observed between Swedish Landrace and Yorkshire breeds (Tables 1 and 2). The same genetic system is probably also operating for other breeds. Preliminary studies have been performed with litters of pure Welsh, Tamworth and Middle White breeds, only one litter of each breed being investigated. The results demonstrated the same range of activity values for these breeds. There were four groups of activity (25, 50, 100, and 125) in the litter of eleven Welsh piglets, the activity of their father and mother being 25 and 125, respectively. We concluded that these phenotypes of the parents corresponded to the genotypes aA_1 and A_1A_4, from which the expected four genotypes of the offspring (aA_1, A_1A_1, aA_4 and A_1A_4), corresponding to the four phenotypes found, could be evaluated. The age of the two other litters were too low for the phenotypes to be conclusively settled. In the Tamworth litter (39 days old), the father of which had activity 20 (probable a low phenotypic value) and the mother 50, there were probably two phenotypes (75 and 125). The father of the Middle White litter had activity 145 and the mother 125; their offspring (36 days old) showed one or two group(s) of esterase activity (100—125). These values were probably too low to represent final phenotypic activities

because of the continuing increase in esterase activity at this age (see Fig. 1).

4. Esterase activity of boars and sows after sexual maturity

All pigs used in the genetic analyses were tested when they had reached the enzyme activity of blood plasma which corresponds to their genotype. The male piglets were castrated at the age of three to four weeks unless otherwise stated. Preliminary investigations, as noted below, showed that the most convenient age for testing litters was from two month to just before sexual maturity, *i.e.*, at six or seven month of age.

After sexual maturity, at least in boars, the arylesterase activity level in plasma seems to be more variable. A number of individual animals of both sexes have been used in studies of this activity for longer (one year) or shorter periods (2—4 months). The results obtained were explanatory for the difficulties which arose in some cases during the early stage of these studies to correlate, on a genetic basis, a found phenotype of certain mature boars with those of his offspring. The activity found for the father was too low to be in conformity with the proposed hypothesis for gene action. For instance, it was demonstrated with a boar of esterase activity 150 before sexual maturity that the activity gradually decreased with increasing age to a value of about 30 and that this low phenotypic activity was then fairly constant. In cross tests (see, *e.g.*, matings Nos. 61 and 62, 66 and 67, etc.) the genotype of such a boar could be evaluated, *e.g.*, by matings with sows of zero activity (genotype *aa*) which resulted in one phenotype (75) in the offspring corresponding to genotype aA_s. The low phenotypic esterase activity of mature boars had no influence on the expected segregation in the progeny. In most boars investigated in these studies, the esterase activity decreased after sexual maturity. We are inclined to suggest that this phenomenon, evidently common to boars, is due to the influence of male sex hormones on the biosynthesis of active protein.

In contradistinction to mature boars, the sows show small variations in plasma arylesterase activity. In some cases sows had higher esterase activity when sexually matured than two to three months before maturity. We observed that the activity was slightly higher briefly before partus, decreased to below the normal value at the time of delivery, increased during the two to three weeks after delivery and then again decreased to normal (*cf.* Fig. 1). It is possible that these variations in

enzyme activity are due to changes in the blood plasma volume during pregnancy. For sows, too, we believe that esterase biosynthesis is influenced by sex hormones. This problem is under investigation.

5. Probable existence of modifying gene action

In a few cases the activity was found to be of a constant higher value than expected and it was so even before and after maturity. Sow "577", for instance, had activity 150—175 and the offspring in a mating (No. 19) with a boar of genotype *aa* gave one group of phenotype 90 ± 10 already at the age of 2—3 months. In a cross test (No. 27) between one of the sows ("1") in this litter with a boar of genotype aA_1 ("426") we could evaluate the genotype of the sow as aA_3. All four expected phenotypes were found in this litter, and it was concluded therefore that their grandmother ("577") was of genotype A_3A_3 in spite of her phenotypic activity being constantly higher than 150. The genotype aA_3 of her daughters ("1", "8", "350", and "356"; see Table 1), despite exhibiting activity 90, were established in further cross tests. The sows "8" (No. 44) and "350" (No. 60) gave normal phenotypes in the offspring. For sow "356" (Nos. 51 and 55), however, the 125 and 150 phenotypes of her offspring also had higher activity than expected.

Sow "413", the genotype of which was established to be A_2A_3 by a number of cross tests (Nos. 14, 15, 33, 63, 64), had activity 150—175. The same activity value was also found for her sister "418". These values were obtained at different stages in the sexual cycle. Similarly, sows of genotype A_2A_3 ("54" and "55", sisters) showed constant esterase activity of 125—150. It will be noted that in no case of constant high phenotypic activity, was the deviation from normal values more than one 25-unit. Whether this high phenotypic activity found for certain sows was influenced by an activating system of a genetic or non-genetic origin could not be settled in the present series of experiments.

IV. DISCUSSION

The results presented above, in our opinion, strongly support the hypothesis that plasma arylesterase in pigs is genetically determined, and is due to the additive effect of a pair of alleles. This genetic system for the synthesis of an active protein is unique, and no similar examples have been reported so far for higher animals. It is, however, possible that *individuality at the biochemical level, regarding inter-individual*

quantitative variation in enzyme efficiencies observed in several normal individuals, might be explained on a similar genetic basis.

The biochemical reactions determining the genetically controlled arylesterase activity in pigs are unknown. It has been demonstrated that the inter-individual differences in activity before maturity is not due to the presence of regulating activators or inhibitors in the blood. The various activity levels observed may be due to the same enzyme protein, the concentration of which varies or the specific activity of which is different in various phenotypes. The first explanation is the most likely, since it would be difficult otherwise to explain the rise in activity for certain groups in a litter during the course of lactation, and also the various activity levels observed. A third possibility is that the various levels are due to the presence of different enzyme molecules of various turnover numbers, but this explanation seems unlikely for the same reasons. We have found no differences in properties of the activity of various phenotypes, and these are not separable by electrophoresis. The increase in esterase activity of piglet plasma during the suckling period goes parallel with the increase in concentration of albumin (to which this enzyme belongs). We therefore suggest as a working hypothesis that the various activity levels found actually reflect various concentrations of the same enzyme protein, the synthesis of which is genetically determined by a series of multiple alleles. This synthesis is probably enzymatically controlled, and the activity of the enzyme(s) involved is (are) directly influenced by gene action.

As far as the general condition and resistance to common diseases are concerned, there were no differences observed between pigs having no arylesterase activity and those with different activity levels.

We have no explanation so far as to the mechanism underlying the formation of the set of alleles producing multiple activity levels. One possible explanation is the occurrence of duplicate, triplicate and quadruplicate genes, corresponding to the alleles A_2, A_3 and A_4. This means that these alleles might be regarded as the result of duplication, etc., of gene A_1 at the same locus in the chromosome.

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SUMMARY

(1) Arylesterase is a type of esterase which splits aromatic esters (*e.g.*, phenyl acetate) at a higher rate than other esters, and is present in the blod plasma of all mammals. Normal pigs exhibit great inter-individual variation, activity values ranging from 0 to 200. The functional significance of these individual differences is not known.

(2) The plasma of new-born animals have no arylesterase activity, irrespective high or medium activity in the plasma of their parents. Differentiation into groups of various arylesterase activities begins on the second to fourth day of life, adult values being reached at the age of about 50 days. This phenomenon is illustrated in the present paper by the results obtained in a back-cross test.

(3) Blood plasma arylesterase in pigs is genetically determined. The various phenotypic activity levels are produced by a set of multiple alleles, each determining a plasma arylesterase activity of 0, 25, 50, 75 and 100 respectively. The distinguishable phenotypes are the manifestation of an additive effect of a pair of these alleles.

(4) This theory for the genetic control of arylesterase formation in pigs is based on the results obtained in back-cross tests and a great number of crosses with pigs of various phenotypes belonging to Swedish Landrace and Yorkshire breeds. This paper presents in detail the analyses of all tested litters and all mating experiments performed (altogether 713 individual animals in 72 matings, including back-crosses and F_2-generations). The phenotypic arylesterase activities of the offspring are illustrated in histograms.

(5) There are no sex differences for the biosynthesis of arylesterase of pig plasma. After sexual maturity the arylesterase activity of boars gradually decreases with increasing age. This low phenotypic esterase activity of mature boars has no influence on the expected segregation in the offspring. It is probably due to the influence of male sex hormones. For sows, too, the activity seems to be influenced by sex hormones.

(6) The probable existence of modifying genes is briefly discussed, as well as are the conceivable biochemical reactions being controlled by the genetic system demonstrated.

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Note added in proof. Results have now been obtained which are consistent with the hypothesis that sex hormones are a possible factor for arylesterase biosynthesis. When a mature boar with a low phenotypic activity of 30 was castrated, the arylesterase activity increased gradually and had reached after three weeks a value (130—140) close to that found before maturity (150). Moreover, this high activity could again be reduced to the low value when 100 mg of testosterone was administered daily intramuscularly; the low value was reached about three weeks after start of hormone treatment. Details of this and similar experiments will be published in *Nature*.

THE FREQUENCY OF XO MALES AND INDUCED AUTOSOMAL CROSSOVERS AFTER IRRADIATION OF *DROSOPHILA MELANOGASTER* MALES IN AIR OR COMMERCIAL NITROGEN

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INTRODUCTION

AFTER irradiation of *Drosophila* males the highest yield of XO males, obtained during the 7th—9th day after treatment, corresponds to cells treated in early meiosis (SÄVHAGEN, 1959). Therefore it would be of interest to study the frequency of induced XO males and, at the same time, induced autosomal crossovers in meiotic and premeiotic stages. The latter observation is especially interesting since AUERBACH (1954) has used the first occurrence of crossing-over and the period of excessive sterility as characteristics for cells treated in the early spermatocyte stage, and clusters of identical mutations as an indication of cells which at the time of treatment were in the spermatogonial stage.

Since THODAY and READ (1947) showed that irradiation under anaerobic conditions reduced the number of cells showing bridges or fragments at anaphase a number of studies have been performed on *Drosophila* concerning the relation between oxygen tension and chromosome breakage and recovery. The main part of these studies have concentrated on mature sperm. However, LÜNING (1958), studying the rate of XO males in the first spermbatch and in the 6th—7th day batches, demonstrated the coexistence of a spontaneous recovery and of differential sensitivity in spermiogenesis in *Drosophila*. The present experiment was therefore also planned in order to investigate the effect of irradiation under anoxia upon the yield of XO males and upon the rate of induced autosomal crossovers during the period of highest sensitivity to X-ray irradiation.

MATERIAL AND METHODS

It is well-known (DEMPSTER, 1941; STRØMNÆS, 1949; BONNIER and LÜNING, 1950; LÜNING, 1952; SÄVHAGEN, 1959, 1960 a; LÜNING, in press) that the response to irradiation depends upon the age of males at treatment, stages in spermiogenesis and the type of mutations studied. In the present experiment the occurrence of XO males has been used as an indication of genetic damage. This technique has been used in a study concerning the pattern of sensitivity to X-ray treatment (SÄVHAGEN, 1961). The method is more thoroughly discussed in that paper. In the same paper the reason for using mass-matings for restricted periods instead of mating the irradiated males individually to virgin females is also given. By use of a dual-purpose stock it is possible to study at the same time the frequency of induced XO males and the occurrence of induced autosomal crossing-over. The importance of scoring the different induced effects in the same stock has already been pointed out by AUERBACH and MOSER (1953) and AUERBACH (1954).

In the present experiment crossing-over was studied in the third chromosome with the following markers: ru (0.0), h (26.5), st (44.0), p^p (48.0), ss (58.5) and e^s (70.7). In order to make it possible to study both losses of paternal sex chromosomes and induced autosomal crossing-over the males had to have a Y chromosome with a marker gene, and as well to be heterozygous for the marker genes in chromosome 3. These requirements are fulfilled by males shown in Fig. 1.

By crossing *yf*:=; sc^8Y ♀♀ with *M5*; sc^8Y ♂♂ (cross 1) it was possible to detect if detachment had occurred in the attached X stock. Females from this stock were crossed to males homozygous for the marker genes, *ru*, *h*, *st*, p^p, *ss*, e^s (cross 2) and from this cross one would obtain males heterozygous for the marker genes in the 3rd chromosome and carrying a sc^8Y chromosome. sc^8Y contained the normal allele for y-locus (y^+). In studies of losses of sex-chromosomes these males were mated to *y w sn* females. The normal offspring were phenotypically wild type females and *w sn* males, while losses of the paternal sex chromosomes gave XO males phenotypically *y w sn* and hence easily detectable.

When, on the other hand, induced crossing-over was to be studied the males were individually mated to females homozygous for the marker genes in chromosome 3. The males were taken out when 0—1 day old and then irradiated when they awoke after etherization. The doses ranged from 700 r to 4400 r (550 r/minute). The flies were irra-

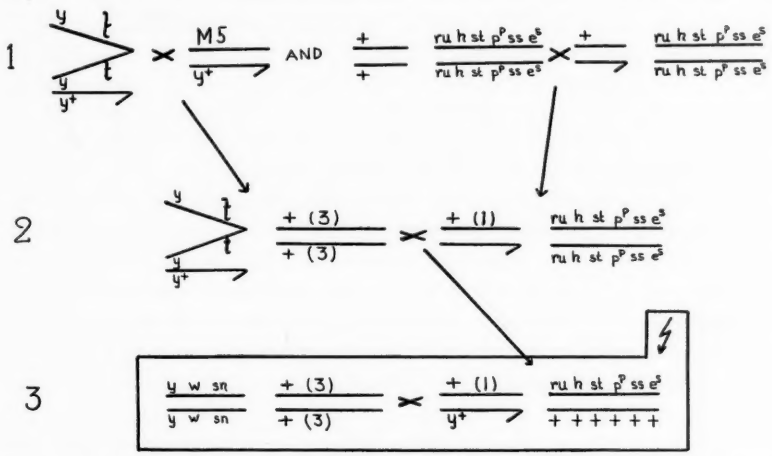

Fig. 1. The different stocks used in order to obtain the dual-purpose male stock, and the experiment cross (3). For further explanation see the text.

diated in a wooden box with a plexiglas top and through the box was passed a stream of air or of commercial nitrogen. In the latter case the males were left 15 minutes in commercial nitrogen before irradiation. The irradiation was performed at room temperature, otherwise stocks and experimental cultures were kept in an incubator at approximately 25° C.

Immediately after irradiation the males were transferred to a mating cage. The first mating period lasted from 0 to 4 hours after irradiation but from the 4th day and onwards every mating period lasted 24 hours. Between the first mating period and the 4th day the irradiated males were kept with an excess of virgin females. At the end of each mating period the males and females were separated. The males were transferred to a new mating cage and new virgin females were introduced. The impregnated females were placed in vials, 5—7 in each. They were allowed to oviposit for three 3-day periods. The vials were emptied on the 12th and the 15th day when the offspring were recorded.

On the 9th day (in some experiments on the 7th day) after irradiation 50 males per series were taken out in order to test the frequency of induced crossing-over in the third chromosome. Each male was mated individually to 2—3 virgin females homozygous for *ru h st p^p ss e^s*. The first mating period consisted of three days, except for the males which

were taken out on the 7th day a.i.,* where the first period lasted 2 days only. The males were then transferred to new virgin homozygous *ru h st* p^p *ss* e^s females for two successive mating periods, all consisting of three days. The impregnated females were allowed to oviposit for three 3-day periods. A number of males died before the mating scheme was completed. These were excluded from the test.

In order further to study the effect of irradiation under anaerobic conditions two experiments were performed in which the dose was subdivided into two parts. The first part consisted of 1100 r in commercial nitrogen with an interval of 15 and 60 minutes respectively in air, whereupon the second part, 700 r in air, was given. By this technique it might be possible to study if the primary change is recoverable. The doses were chosen according to LÜNING and HENZE's (1957 c) findings.

RESULTS

The data from studies of losses of paternal sex chromosomes, by means of the occurrence of *y w sn* males in the offspring, is presented in Table 1. Each series consisted of several repeats. The heterogeneity was analysed by χ^2-tests. In the first four mating periods (0—4 hours to the 6th day a.i.) only two cases of heterogeneity were observed. On the other hand during the 7th—9th day a.i., there was a marked heterogeneity in series with irradiation in air. This coincides with the period of highest sensitivity.

The changes in the rate of XO males in successive mating periods are in agreement with experiments of a similar kind in which males of another type were used (SÄVHAGEN, 1959).

As regards the effect of anoxia in different stages, information is also available in Table 1. It is seen that there is a significantly lower rate of induced XO males in experiments performed under anaerobic conditions compared to those irradiated in air. The difference is most drastic in mating periods corresponding to the highest sensitivity (Ser. C vs Ser. E). This is also evident from comparisons between Series C and G. 4400 r in N_2 (Ser. G) gives a higher rate of XO males in the first mating periods (up to the 6th day a.i.) than 1100 r in air (Ser. C) but the relationship is reversed in broods from the 7th and the 8th day a.i. (Ser. G$<$Ser. C). From Fig. 2 and Table 1 it is furthermore obvious that the changes in the rate of induced XO males in the N_2-series parallel that of XO males obtained after irradiation in air. Thus one may

* Henceforth the abbreviation a.i. will be used for after irradiation.

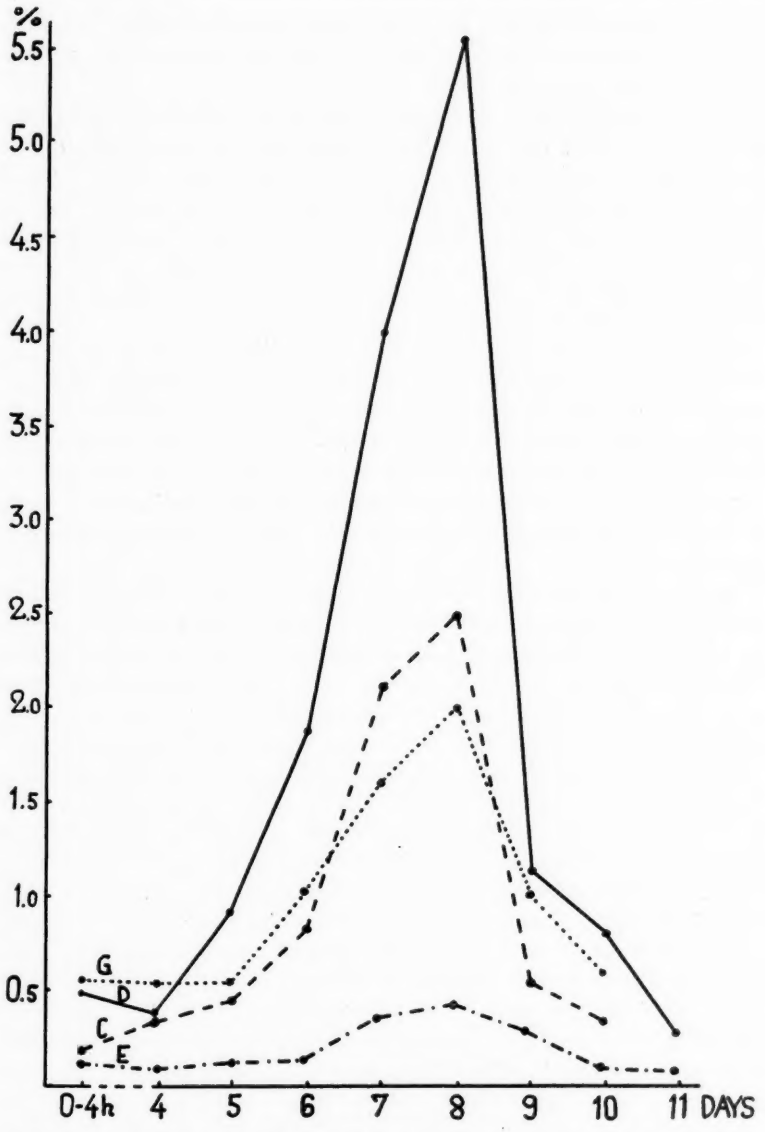

Fig. 2. The frequency of induced XO males in successive mating periods after treatment with 1100 r in air (Ser. C), 1800 r in air (Ser. D), 1100 r in N_2 (Ser. E) and 4400 r in N_2 (Ser. G).

conclude that pretreatment for 15 minutes and the immediately following irradiation in nitrogen atmosphere does not influence the meiotic activity in any gross way.

Sterility and decrease in fertility has been considered (WELSHONS and RUSSEL, 1957) as one of the consequences of irradiation of late spermatogonia and early spermatocytes. From Table 1 a it is evident that in mating periods with the highest rate of XO males, there is also the smallest number of offspring. In succeeding days there is, parallel to a decrease in XO males, an increase in the number of offspring (Tables 1 a and b). In series irradiated at anoxia the change in fertility was obvious only at the highest dose level.

It is known from studies by LÜNING *et al.* (1957 a, b, c, 1958) and LÜNING (1958 a, b) that there exists a recovery phenomenon. This has been demonstrated through a series of experiments on mature spermatozoa in which they used different whole-doses in air and nitrogen and doses divided into two fractions, one of which was given in air and the other at anoxia. In the present experiment an attempt has been made to study the recovery phenomenon in cells treated during spermio- and spermatogenesis.

LÜNING and HENRIKSSON (1959), who studied induced recessive lethals in mature spermatozoa observed that up to 30 minutes after irradiation in nitrogen the recovery could be blocked. Thus the first fraction, 1100 r, was in the present experiment, given in nitrogen atmosphere, and, after 15 and 60 minutes respectively in air, the males were irradiated with 700 r in air. The results from these fractionated treatments (Series H and I) are given in Table 2. The figures are the sum of several repeats. The heterogeneity was tested in the same way as in Series A—G in Table 1. It is evident that during the sensitive period (5th—9th day a.i.) there is a consistent difference between Series H and I indicating that an interval of 60 minutes between the two treatments gives a lower rate of XO males than is the case when the interval is only 15 minutes.

In order to facilitate comparisons, the rates of XO males in the relevant series from Tables 1 and 2, are presented in Table 3. From this it is seen that summing the two separate treatments, 1100 r in N_2 (Series E) and 700 r in air (Series B), gives rates of about the same order of magnitude as in Series I in which the two dosages were given to the same males with an interval of 60 minutes. Hence, with a dose-interval of 60 minutes the recovery process seems to be finished. On the other hand, when the interval is 15 minutes (Series H) the rates of XO males during the 6th—8th day a.i. were higher than the sum of Series B+E,

TABLE 1. *0—1 day old $y^+ w^+ sn^+$; sc^8Y males (heterozygous for the marker genes in the 3rd chromosome) irradiated in air or in nitrogen atmosphere and mated to y w sn females. Frequency of XO males and total number of offspring (n) in successive mating periods. * = heterogeneity within the material ($P < 0.05$).*

Table 1 a.

Series	Dose	Atmosphere	Time after irradiation															
			0—4 hrs		4th day		5th day		6th day		7th day		8th day		9th day		10th day	
			n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂
A	Control	Air	27,958	0.07	43,995	0.08	54,712	0.04	54,096	0.04	38,380	0.03	52,464	0.02	40,460	0.05	13,254	0.03
B	700 r	„	55,523	0.19	—	—	—	—	69,502	0.49	47,642	0.76	45,751	1.32*	38,377	1.08*	36,823	0.21
C	1100 r	„	15,316	0.19	36,778	0.28*	23,290	0.44	25,355	0.84	13,371	2.11*	9,658	2.52	11,674	0.58	14,432	0.36
D	1800 r	„	30,790	0.48	39,311	0.36	19,491	0.90	12,822	1.87	8,337	4.00*	2,983	5.63*	13,582	1.14*	18,067	0.82
E	1100 r	N_2	35,611	0.13	37,292	0.09	44,390	0.14	44,850	0.12	41,606	0.37	43,621	0.43	31,230	0.30	38,284	0.10
F	1800 r	„	20,985	0.18	49,243	0.17	52,127	0.17	37,738	0.24	43,793	0.46	48,949	0.62	35,085	0.63	33,776	0.20
G	4400 r	„	11,722	0.55	40,054	0.52	25,536	0.53	20,681	1.05*	14,725	1.60	15,133	2.0	10,241	1.03	29,304	0.60

Table 1 b.

		XO♂♂	n	% XO♂♂
Ser. D	11th day	38	13,575	0.28*
	12th „	16	17,425	0.09
	13th „	15	16,205	0.09
	14th „	7	14,274	0.05
	15th „	10	15,606	0.06
	16th „	4	12,566	0.03

TABLE 2. *0—1 day old $y^+ w^+ sn^+$; sc^8Y males (heterozygous for the marker genes in the 3rd chromosome) given 1100 r in commercial nitrogen, after an interval of 15 or 60 minutes in air irradiated with 700 r in air and immediately mated to y w sn females. Frequency of XO-males and total number of offspring (n) in successive mating periods. * = heterogeneity within the material ($P < 0.05$).*

Series	Dose and atmosphere	Time after irradiation													
		0—4 hrs		4th day		5th day		6th day		7th day		8th day		9th day		10th day	
		n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂	n	% XO♂♂
H	1100r N_2 — 15 min. air — 700 r air	9,615	0.22	45,131	0.31	35,357	0,64	36,255	1.23	27,110	2.26*	17,680	2.49*	9,281	1.22*	14,282	0.34
I	1100 r N_2 — 60 min. air — 700 r air	7,777	0.35	25,170	0.18	13,865	0.21	9,427	0.60	8,710	1.66	8,939	1.31	4,035	0.67	9,501	0.42

TABLE 3. *Comparison between the rate of induced XO males after a dose in air (1800 r, Ser. D), or in nitrogen (1800 r, Ser. F), two dose-fraction experiments (Ser. H and I, Table 3) and the combined data for: 1100 r in N_2 (Ser. E) and 700 r in air (Ser. B) corrected for the mean of the control (0.05 %, Ser. A), hence (Ser. B+Ser. E—Ser. A).*
**=heterogeneity within the material ($P<0.05$).*

Series	Dose and atmosphere	The frequency of XO males in different mating periods a. i.				
		6th day	7th day	8th day	9th day	10th day
D	1800 r in air	1.87 %	4.00 %*	5.63 %*	1.14 %*	0.82 %
H	1100 r N_2 — 15 min air — 700 r air	1.23 %	2.26 %*	2.49 %*	1.22 %*	0.34 %
I	1100 r N_2 — 60 min air — 700 r air	0.60 %	1.66 %	1.31 %	0.67 %	0.42 %
B+E−A	1100 r N_2 + 700 r air (combined data from Table 1)	0.56 %	1.08 %	1.70 %	1.33 %	0.26 %
F	1800 r N_2	0.24 %	0.46 %	0.62 %	0.63 %	0.20 %

but they are distinctly lower than in Series D in which the dose was given as one single treatment in air. These results indicate that there is some recovery after irradiation of the sensitive stages but that it could be only partly blocked by 700 r given in air within 15 minutes.

The experiment was planned to measure simultaneously the frequency of induced XO males and induced crossing-over in the third chromosome after irradiation of *Drosophila* males with different X-ray doses in air or in commercial nitrogen. Individuals showing at least one but not all of the six marker genes *ru*, *h*, *st*, p^p, *ss*, e^s were recorded. Cases showing two or more marker genes were classified as recombinants. Those showing only one marker gene could as well be due to recombination as to mutation or deficiency. In order to avoid an overestimation, flies showing only one marker gene are not included in Table 4 (a, b, c) unless it was possible to find the complementary class.

The crossover data obtained after irradiation in air is shown in Table 4 a. For the first mating period (7th—8th day after treatment) data is only available for 700 r. Here one finds only single crossovers

TABLE 4 a. *Frequency of recombinants (r) and total offspring (n) in successive mating periods from X-ray irradiated, 0—1 day old males, heterozygous for the marker genes: ru, h, st, p^p, ss, e^s. Irradiation in air. *=recombinants occurring in the progeny from the same male.*

Series	Dose in r	Days after irradiation							
		7th—8th		9th—11th		12th—14th		15th—14th	
		n	r	n	r	n	r	n	r
D	1800			3615	1 *ru h* 5 *ru h st* 5 *p^p ss e^s* 2 *ru h st p^p* 1 *ru h st p^p ss* 3 *p^p ss e^s* * 1 *ru h st*; 1 *p^p ss e^s* * 1 *ru h st*; 1 *ss e^s* * 1 *ru h st p^p ss*; 1 *e^s* * 1 *ru h st*; 1 *ru h st p^p* * 1 *p^p ss e^s*; 1 *ss e^s* *	3317	1 *ru h st p^p* 1 *p^p ss e^s* 8 *p^p ss e^s* * 3 *ru h st*; 2 *p^p ss e^s* * 7 *ru h st*; 3 *p^p ss e^s* * 2 *ru h st*; 1 *p^p ss e^s* *	3177	1 *ru h st* 1 *ru h st p^p*; 1 *ss e^s* *
C	1100			4491	3 *ru h st* 1 *ru h st p^p* 1 *ru h*; 1 *p^p ss e^s* * 1 *ru h st p^p*; 1 *ss e^s* * 1 *ru h*; 1 *ru h st* * 1 *ru h st*; 1 *p^p ss e^s* *	5491	2 *ru h st* * 2 *ru h st* * 4 *ru h st* * 11 *ru h st*; 11 *p^p ss e^s* * 1 *ru h st*; 1 *p^p ss e^s* * 2 *ru h st* 6 *p^p ss e^s* 4 *ss e^s* *	4288	41 *ru h st*; 19 *p^p ss e^s*; 2 *ru h* * 2 *ru*; 9 *h st p^p ss e^s*; 4 *p^p ss e^s* * 10 *ru h st*; 7 *p^p ss e^s* *
	700	1701	1 *ru h st p^p* 1 *p^p ss e^s*	1783	1 *ru h st p^p* 1 *p^p ss e^s*	4927	1 *ru h* 2 *ru h st*	2584	2 *h st p^p ss e^s* *

					1 *ru h st* 1 *ru h st*; 1 *pp ss es* *		2 *ru h st* 6 *pp ss es* 4 *ss es* *		7 *pp ss es* *
B	700	1701	1 *ru h st pp* 1 *pp ss es*	1783	1 *ru h st pp* 1 *pp ss es* 1 *ru h*; 1 *pp ss es* *	4927	1 *ru h* 2 *ru h st* 2 *pp ss es* * 1 *ru h st*; 1 *ru h st pp* * 1 *ru h st*; 1 *pp ss es* *	2584	2 *h st pp ss es* *

TABLE 4 b. *Frequency of recombinants (r) and total offspring (n) in successive mating periods from X-ray irradiated males, heterozygous for the marker genes: ru, h, st, p^p, ss, e^s. Irradiation in nitrogen atmosphere. *=recombinants occurring in the progeny from the same male.*

Series	Dose in r	Days after irradiation							
		7th—8th		9th—11th		12th—14th		15th—17th	
		n	r	n	r	n	r	n	r
G	4400	1743	2 *pp ss es*	2173	4 *ru h st* 4 *pp ss es* 1 *ru h st*; 1 *pp ss es* *	4273	2 *ru h st* 1 *pp ss es* 2 *pp ss es* * 21 *pp ss es* * 6 *ru h st pp ss*; 3 *es* * 4 *ru h st*; 2 *pp ss es* * 10 *ru h st*; 8 *pp ss es* * 12 *ru h st*; 14 *pp ss es* *	4747	2 *ru h st* 3 *ru h st pp* * 4 *pp ss es* 1 *ru h st*; 1 *pp ss es* * 8 *ru h st*; 7 *pp ss es* * 20 *ru h st* 26 *pp ss es* 1 *ru h st pp ss* 4 *es* *
F	1800			4486	2 *ru h* 2 *ss es* 2 *pp ss es* 1 *st pp ss es*	3291	1 *ru h st pp ss* 1 *pp ss es*; 1 *ss es* *	2171	—
E	1100			3260	—	3939	1 *ru h st pp*	6044	1 *ru h st* 1 *h st pp ss es*

TABLE 4 c. *Frequency of recombinants (r) and total offspring (n) in successive mating periods from X-ray irradiated males, heterozygous for the marker genes: ru, h, st, p^p, ss, e^s. Dose divided into two parts: 1100 r in nitrogen atmosphere and, after an interval of 15 (60) minutes, 700 r in air.*
** = recombinants occurring in the progeny from the same male.*

Series	Dose	Days after irradiation: 7th–8th		9th–11th		12th–14th		15th–17th	
		n	r	n	r	n	r	n	r
I	1100 r N_2 — 60 min air — 700 r air	3360	1 *ru h st* 2 *pp ss* e^s 1 *ru h st pp ss* 1 *st pp ss* e^s 2 *pp ss* e^s * 1 *ru h st*; 1 *pp ss* e^s *	3278	2 *ru h st* 1 *pp ss* e^s 1 *ru h st*; 1 *pp ss* e^s *	4446	4 *ru h st* 1 *pp ss* e^s 1 *ru h st pp* 2 *pp ss* e^s * 2 *ru h st* * 1 *ru h st*; 1 *pp ss* e^s * 1 *ru h st*; 1 *pp ss* e^s * 1 *ru h st*; 1 *pp ss* e^s * 1 *ru h st*; 1 *pp ss* e^s; 1 *ss* e^s * 2 *ru h st*; 1 *pp ss* e^s; 1 *ru h st pp*; 1 *pp ss* * 1 *pp ss*	4254	1 *pp ss* e^s 4 *pp ss* e^s * 8 *ru h st*; 6 *pp ss* e^s * 5 *ru h st* * 6 *ru h* 2 *st pp ss* e^s *
H	1100 r N_2 — 15 min air — 700 r air	1257	1 *ru h st*	3799	3 *ru h st* 2 *ru h st pp* 1 *ru h st pp ss* 3 *pp ss* e^s 1 *st pp ss* e^s 2 *pp ss* e^s * 1 *ru h st*; 2 *pp ss* e^s * 1 *ru h st*;	4696	2 *ru h st* 1 *ru h st pp* 1 *pp ss* e^s 1 *ru h st pp*; 2 *h st pp ss* e^s * 3 *ru h st*; 1 *pp ss* e^s *	3202	1 *ru h st pp* 3 *pp ss* e^s * 2 *ru h st*; 6 *pp ss* e^s *

which is in accordance with the results obtained by FRIESEN (1936, 1937) who states that meiotically produced crossovers occur singly during the period of low fertility. The second mating period (9th—11th day after treatment) mostly yields recombinants occurring singly but at the same time in rare cases bundles of identical or complementary crossovers are obtained. According to FRIESEN (1937) bundles of identical and complementary crossovers are a sign of crossovers induced in spermatogonia. Thus, this mating period might correspond to cells treated chiefly as early spermatocytes and a few treated as late spermatogonia.

From the second mating period and beyond, data is available for treatments with different X-ray doses. However, the above mentioned result is obtained independent of the dose given. There exists, of course, a dose dependence if one compares the frequency of induced recombinants. In order to obtain a more exact analysis of this material, a rendering of the data for each male individually is demanded, but the present material is too small for such an analysis.

In the third mating period (12th—14th day after treatment) one finds clusters of identical and complementary crossovers and only a few single recombinants (Ser. C and D). From this one may conclude that the spermatozoa available for insemination during these days might chiefly have been treated as spermatogonia. This becomes even more evident from the observations of the frequency of induced recombinants in the subsequent mating period from the 15th to the 17th day after treatment (Ser. C). In this mating period the same indication of spermatogonial crossovers is observed. The absence of clusters of crossovers during the 4th mating period in Series D might be due to the small material. It is also uncertain whether the occurrence of the two recombinants in Series B during the 15th—17th day after irradiation is a sign of clustering or represents two independent crossovers occurring in different cells. The obtained crossover data in Table 4 a fits well with the sensitivity pattern obtained through XO studies (Table 1), in which the spermatozoa used during the 7th to the 9th day after irradiation correspond to cells treated at early meiotic stages (SÄVHAGEN, 1960 a).

The frequency of induced crossovers after X-ray treatment under anaerobic conditions is seen in Table 4 b. From the data obtained it may be seen that pretreatment with commercial nitrogen and irradiation under anaerobic conditions reduces the amount of induced recombinants. Thus 1100 r in N_2 (Ser. E) and 1800 r in N_2 (Ser. F) yielded very few crossovers and only single recombinants. The absence of

TABLE 4 d. *Examples of observed single recombinants, clusters of identical and complementary crossover classes in successive mating periods from single males irradiated with 1100 r in air (Ser. C) and 4400 r in N_2 (Ser. G).*

Series	♂ nr	Time after irradiation					
		9th—11th day		12th—14th day		15th—17th day	
		n	r	n	r	n	r
C	1	183	—	230	11 *ru h st*; 11 *p^p ss e^s*	169	—
	25	68	1 *ru h st*	285	2 *ru h st*	175	41 *ru h st*; 19 *p^p ss e^s* 2 *ru h*
	62	30	—	233	2 *ru h st*	144	10 *ru h st*; 7 *p^p ss e^s*
G	6	92	1 *ru h st*; 1 *p^p ss e^s*	151	4 *ru h st*; 2 *p^p ss e^s*	165	—
	20	52	—	146	10 *ru h st*; 8 *p^p ss e^s*	85	1 *ru h st*; 1 *p^p ss e^s*
	22	107	1 *ru h st*	168	12 *ru h st*; 14 *p^p ss e^s*	149	8 *ru h st*; 7 *p^p ss e^s*

cluster formation in Series E and F is evident. This might be due to the small material or perhaps be an effect of recovery.

Turning now to 4400 r N_2 (Ser. G) one finds mostly single recombinants in the first two mating periods (7th—8th day and 9th—11th day a.i.) but from the third mating period there appears clusters. Thus single recombinants and clusters appear in corresponding mating periods in Series G and C (Table 4 d). In Table 4 c the crossover data for Series H and I is seen. From the present material it is not possible to draw any conclusions concerning the effect of dividing the dose into two fractions. Such an analysis has to be done between single males, this becomes even more evident since the material shows an increasing heterogeneity in successive mating periods.

DISCUSSION

That the peak in the rate of induced XO males is obtained during the 7th—9th day after irradiation and that this peak in sensitivity corresponds to cells treated in early meiosis has been shown by SÄVHAGEN (1960 a). This conclusion is based on the occurrence of induced non-disjunction between the paternal X and Y chromosomes. The rate of induced XO males in the present study parallels fairly well the above mentioned results and it may hence be concluded that, even for these

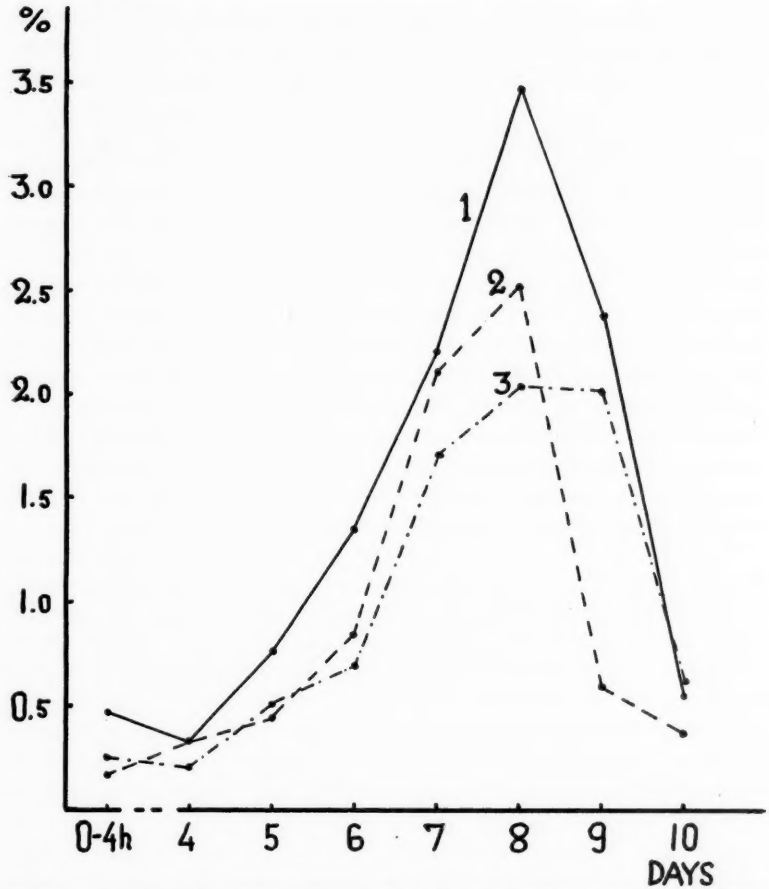

Fig. 3. A comparison between the frequency of induced genetic damages in different stocks after X-ray irradiation with 1100 r in air. 1) per cent XO males after treatment of y^{1a}; sc^{8}Y males (SÄVHAGEN, 1960 a), 2) per cent XO males in the present study (Ser. C) and 3) per cent $\widehat{XX}$O females after treatment of y^{1a}; sc^{8}Y males (SÄVHAGEN, 1960 b).

males, the spermatozoa becoming available for insemination during the 7th—9th day after treatment must correspond to cells treated during early meiosis (prior to anaphase I).

Since AUERBACH (1954) points out that "sperm utilized on a given day or during a given mating period after treatment may be derived from

quite different stages of treated germ cells, depending on the genotypes of the treated ♂♂ and their mates" it is of especial interest to make a comparison between the present results and experiments performed with other stocks.

In a study concerning the rate of induced XO males and non-disjunction females *y w sn* ♀♀ were crossed to irradiated y^{1e}; sc^{8}Y ♂♂ (SÄVHAGEN, 1960 a); in the present study *y w sn* ♀♀ are crossed to irradiated y^{+} w^{+} sn^{+}; sc^{8}Y ♂♂ heterozygous for the third chromosome markers: *ru, h, st,* p^{p}, *ss,* e^{s} and in a study concerning the rate of induced $\widehat{XX}$O females and translocations *y f*:=; sc^{8}Y; *cn bw*; *e* ♀♀ were crossed to irradiated y^{1e}; sc^{8}Y ♂♂ (SÄVHAGEN, 1960 b). Thus the first two experiments differ in the male stock; and the third experiment differs from the first study in the female stock and from the present experiment in both the female and male stocks. From all these experiments it is evident that the peak in the rate of induced XO males and $\widehat{XX}$O females is obtained during the same mating period (Fig. 3). Hence, it is seen that for the above mentioned stocks the products from cells irradiated in early meiotic stages are utilized in the same mating period a.i.

Since the spermatozoa becoming available for insemination during the 7th—9th day after treatment correspond to cells treated during early meiosis it is interesting to compare the frequency of induced XO males (Table 1) and the occurrence of induced crossovers (Table 4). AUERBACH (1954) has used the period of excessive sterility and first occurrence of crossovers as a sign of meiosis. From Table 1 it is seen that after irradiation in air a marked decrease in fecundity is observed during the 7th—9th day after treatment. Turning now to Table 4 a it is seen that, during the 7th—8th day a.i., only single crossovers are observed. Thus the present results are in agreement with AUERBACH's observations. A strong reduction in fecundity during the 8th—9th day after treatment is reported by IVES and RICHMAN (1958) and IVES (1960). FRIESEN (1937) found that the sterility during the 7th—8th day after treatment was due to lack of sperm (confirmed by AUERBACH, 1954) and that this could be taken as a border to the gonial stage. FRIESEN (1936, 1937) furthermore suggested that single crossovers were obtained from cells treated during meiosis, while complementary crossovers and bundles of identical crossovers could be taken as an indication of cells treated as spermatogonia. From Table 4 a and b it is seen that the 9th—11th day after treatment mostly yield single recombinants and a few complementary crossovers; while the following mating periods yield clusters of crossovers. IVES (1960) reports that clusters of reci-

procal crossovers are observed on the 13th day after treatment. SOBELS (1956) working with formaldehyde also observed clustering of crossovers during the 10th—13th day after treatment of the males.

The mating periods corresponding to the 9th—11th day after irradiation seem to constitute a mixture of late spermatogonia and early spermatocytes. However, IVES (1960) has used the recovery of fertility as a sign of treated spermatogonia. He also observed a rise in fecundity on the 11th day after treatment. Turning to Tables 1 a and 1 b it is seen that from the 11th day there is an upward trend in fecundity which might be taken as a further sign of treated spermatogonia.

When examining the frequency of induced XO males it is seen from Tables 1 a and 1 b that on the 14th day after treatment the frequency of induced XO males does not differ statistically from the control value. Thus, it might be taken as an indication that spermatogonia are less sensitive, or that cells with damages leading to losses of the X and/or Y chromosome are eliminated. The observed low rate of induced losses of sex chromosomes in spermatogonia is consistent with observations made by AUERBACH (1954), OSTER (1958), IVES (1960), and BATEMAN and CHANDLEY (1959).

When studying the distribution of crossovers in the third chromosome it is seen that most of the obtained crossovers are localized around the spindle fibre region. This is consistent with observations made by FRIESEN (1936), WHITTINGHILL (1951) and HERSKOWITZ and ABRAHAMSON (1957).

FRITZ-NIGGLI (1958, 1959) observed a greater discrepancy in the yield of dominant lethals in the sensitive period than in spermatozoa when comparing the effect of irradiation in air versus irradiation in nitrogen atmosphere. The present study of induction of XO males confirms these results. It would indicate that the spermatocytes should be more saturated with oxygen than the mature spermatozoa and that part of the difference in sensitivity between spermatocytes and spermatozoa irradiated in air should be due to a difference in the oxygen concentration. On the other hand, the results show that this cannot be the only reason for this difference as there is a significant difference between the mating periods following irradiation in anoxia.

However, when discussing the above related strong influence of low oxygen concentration during the 7th—9th day after treatment, one must remember that, especially during this period, several factors might exert influence upon the results obtained. Among them could be mentioned the occurrence of induced non-disjunction between the paternal

X and Y chromosomes; which, according to SÄVHAGEN (1961), is a factor which increases the rate of XO males during this period. It is possible that anoxia exerts differently strong influences upon induction of chromosome breaks and non-disjunction or other factors leading to losses of the X and/or Y chromosomes. The existence of such a difference has also been demonstrated by SÄVHAGEN (unpublished), who observed that irradiation under anaerobic conditions gives an almost total protection against induced non-disjunction.

That the reduced effect of irradiation under anoxia is not limited to XO males is obvious from the crossing-over study. However, the material is too small to permit any further discussion of the results obtained after treatment in nitrogen atmosphere versus irradiation in air.

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SUMMARY

(1) The present study was performed in order to investigate the frequency of induced XO males and at the same time induced autosomal crossovers in meiotic and premeiotic stages after treatment of 0—1 day old males. The effect of irradiation under anoxia upon the yield of XO males and upon the rate of induced autosomal crossovers was also studied during the period of highest sensitivity to X-ray irradiation. By use of a dual-purpose stock it was possible to study both aberrations in the same stock.

(2) The changes in the rate of XO males in successive mating periods are in agreement with experiments of a similar kind where males of another type have been used (SÄVHAGEN, 1960 a).

(3) It is shown that there is a significantly lower rate of induced XO males in experiments performed under anaerobic conditions compared to those irradiated in air. It is furthermore obvious that the difference is most drastic in mating periods corresponding to the highest sensitivity.

(4) In order further to study the effect of irradiation under anaerobic conditions two experiments were performed where the dose was divided into two parts. The first part consisted of 1100 r in commercial nitrogen with an interval of 15 and 60 minutes respectively in air, whereupon the second part, 700 r in air, was given. The results indicate that there

is some recovery after irradiation of the sensitive stages but that it could be only partly blocked by 700 r given in air within 15 minutes.

(5) That the reduced effect of irradiation under anoxia is not limited to XO males is obvious from the crossing-over study.

(6) The observed crossover data fits well with the sensitivity pattern obtained through XO studies. Hence one may conclude that the present crossover study confirms the results obtained by SÄVHAGEN (1960 a). Furthermore it is evident that spermatozoa available for insemination on the 12th day and onwards chiefly might correspond to cells treated as spermatogonia.

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THE RELATION BETWEEN X-RAY SENSITIVITY AND STAGES OF DEVELOPMENT OF TREATED CELLS IN SPERMATO- AND SPERMIOGENESIS OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

IN mutation work with X-rays and chemical mutagens, several attempts with various indirect methods have been made to correlate the sensitivity period with the treated cell stages. That the age of the males and the length of time between irradiation and fertilization is of importance for the yield of mutations was shown by BONNIER and LÜNING (1950). LÜNING (1952 a, b and c) found that a much higher rate of dominant lethals, recessive lethals and hyperploid males was induced in sperm which become available for insemination 7—10 days after irradiation than in those used 1—6 days after treatment. An explanation of this difference may, according to LÜNING, be that more chromosome breaks are induced in cells ready for insemination 7—10 days after treatment (possibly spermatids) than in mature spermatozoa.

Many investigators have suggested a sensitivity pattern for the cells treated during spermato- and spermiogenesis. Much disagreement still exists as to which stages of the developmental cycle are stages of high sensitivity to chromosome breakage and which are relatively resistant. The present investigation represents an attempt to obtain additional information on the course of meiosis, or, when sperm, which at the time of treatment were in meiosis, are ejaculated. At the same time an attempt is made to correlate this information with a sensitivity pattern.

MATERIAL AND METHODS

In the studies of the effect of X-rays on the production of non-disjunction in *Drosophila melanogaster* females, MAVOR (1921, 1922, 1924),

showed that X-irradiation affected the germ cells and that the effect produced was identical with primary non-disjunction. MAVOR also concluded that an effect of X-rays when applied to eggs during maturation is to cause non-disjunction. By analogy one may suppose that the same process may also be induced by X-irradiation of the males. The occurrence of an increase in the frequency of non-disjunction in sperm ejaculated during a limited period after irradiation must be taken as an indication that those spermatozoa were in meiosis at the time of treatment.

In the choice of experimental stocks much care must be taken in order to ensure the possibility to distinguish phenotypically between paternal and maternal non-disjunction. Besides this, both the females as well as the males, must be guaranteed free from disturbing extra Y-chromosomes. The following stocks were used: the females were taken from a *y w sn* ♀♀×*y w sn*; *sc*^{8}Y ♂♂ stock, and the males from a *y*^{16} ♀♀×*y*^{16}; *sc*^{8}Y ♂♂ stock. *sc*^{8}Y contains the normal allele for *y*-locus (*y*$^{+}$) and on account of this the males in the above mentioned stocks have wild type body colour. By chosing yellow females and grey males in the stocks, one will have a maximum guarantee that these animals are XX and XY respectively. In the experiments *y w sn* females were crossed to irradiated *y*^{16}; *sc*^{8}Y males (Fig. 1). The normal offspring from this cross will be *y* females and *w sn* males. There appeared also some *w sn* females due to maternal non-disjunction. However, these individuals are not recorded.

Of special interest is the occurrence among the offspring of wild type females and yellow males (*y w sn* ♂♂), Fig. 1. The wild type females may be the result of induced non-disjunction between the paternal X- and Y-chromosomes (*y w sn* / *y*^{16} *w*$^{+}$ *sn*$^{+}$; *sc*^{8}Y) but may also originate from induced crossing-over between the X- and Y-chromosomes (possibly *y w sn* / *y*^{16} *w*$^{+}$ *sn*$^{+}$·*y*$^{+}$). In order to distinguish between these two possibilities each wild type female has to be tested individually by crossing them to *y w sn*; *sc*^{8}Y males. If the wild type females are due to induced crossing-over there will appear two classes of non-recombinant females (Fig. 2 a) while there will be four non-recombinant classes in offspring from non-disjunction females (Fig. 2 b). *y w sn* males on the other hand result from fertilization with spermatozoa which have lost the X and/or *sc*^{8}Y or at least the marker gene in the *sc*^{8}Y chromosome (WOLFF and LINDSLEY, 1960). These individuals are supposed to be XO males and may be the result of: a) elimination of X and/or Y chromosome caused by simple breaks or reunion of the

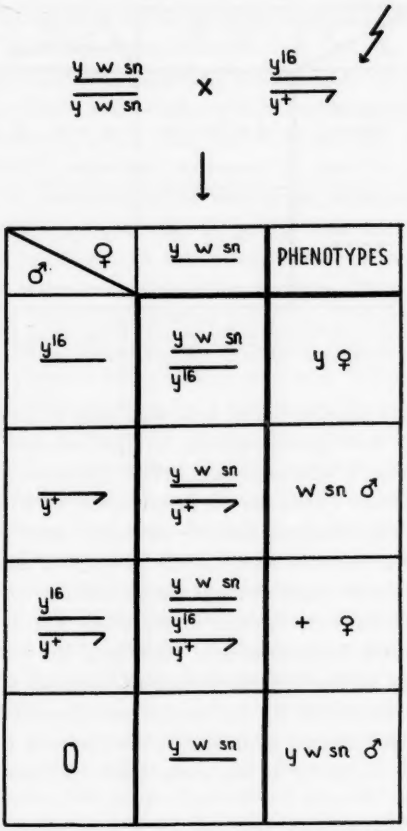

Fig. 1. The offspring from matings *y w sn* ♀♀×y^{16}; sc^8Y ♂♂.

broken ends to form dicentric and acentric chromosomes (MULLER, 1940), b) disturbances in the spindle structure (non-disjunction), c) disturbances in the DNA-synthesis (stickiness) or other physiological and mechanical factors which may influence the result. These possibilities will be further discussed in another place in this paper. The *y w sn* males may also be due to mutation in sc^8-locus, but these males should be fertile.

The absence of a Y chromosome in *y w sn* males can be further verified by testing the presumed XO males individually for fertility since BRIDGES (1916), and SAFIR (1920), have shown that males lacking the

♂ \ ♀	y w sn	$y^{16} w^{+} sn^{+}$ / y^{+}
y w sn	y w sn ♀	+ + + ♀
y^{+}	+ w sn ♂	+ + + ♂

Fig. 2 a. The offspring (non-recombinant classes) from tested wild type females due to induced crossing-over between the paternal X and Y chromosomes.

Y chromosome are sterile. SAFIR was also able to prove by cytological examination that the spermatogonia in the obtained XO males contained an unpaired X chromosome. That spermatozoa lacking the X or Y chromosome are viable was demonstrated by KUHN (1930).

In the present experiments 150 *y w sn* males were tested for fertility. Only 2 were fertile the rest were sterile in spite of being transferred to new females and new vials several times. Hence, there seemed to be reason to suppose that the *y w sn* males were XO or at least had lost parts of the paternal Y-chromosome, including the marker gene y^{+}.

The importance of scoring the different induced effects in the same stock has been pointed out by AUERBACH and MOSER (1953) and AUERBACH (1954). In the present experiment the use of a dual-purpose stock makes it possible to study at the same time the frequency of induced

♂ \ ♀	y w sn	y^{16} + + / y^{+}	y^{16} + +	y w sn / y^{+}
y w sn	y w sn ♀	+ + + ♀	y + + ♀	+ w sn ♀
y^{+}	+ w sn ♂	+ + + ♂	+ + + ♂	+ w sn ♂

Fig. 2 b. The offspring (non-recombinant classes) from tested wild type females due to induced non-disjunction between the paternal X and Y chromosomes.

XO males and the occurrence of induced non-disjunction between the paternal X- and Y-chromosomes.

Usually dominant lethals, generally presumed to be caused by breaks, which remain unjoined, and breaks which rejoin in asymmetrical interchanges (PONTECORVO, 1942; LEA and CATCHESIDE, 1945), have been used as indication of radiation damages. To use the percentage of unhatched eggs as a measurement of dominant lethality is only correct, as AUERBACH (1954) pointed out, if one is able to show that all unhatched eggs are fertilized. In this study the occurrence of XO males has been used as indication of genetic damage. This method was presented by MULLER (1940) for testing the rate of viable losses of X and Y chromosomes. The technique has been used for ring-shaped chromosomes by BAUER (1939, 1942), BAKER and VON HALLE (1955) and for both rod and ring-chromosomes by LÜNING and HANNERZ (1957).

DEMPSTER (1941), STRØMNÆS (1949), BONNIER and LÜNING (1950), and LÜNING (1952 b) showed that the difference in sensitivity (yield of mutations) depended upon the age of the males at irradiation. On the basis of these findings 0—1 day old and 3—4 day old males were irradiated and tested. Besides the age of the males at irradiation it is necessary to delimit the mating periods in relation to irradiation. BAKER and VON HALLE (1953) showed that there were induced more dominant lethals in the sperm ejaculated 0—24 hours after irradiation than 24—48 hours after irradiation. These findings have been further investigated by LÜNING (in press), who was able to demonstrate the existence of changes in the rate of induced mutations in spermatozoa released earlier than 24 hours after irradiation. He also observed that this change did not occur at the same time in 0—1 and 3—4 day old males. This difference between 0—1 day and 3—4 day old males makes it necessary to compare the frequency of induced XO males in mature spermatozoa only during 0—4 hours after irradiation.

An experiment with the present aim may be done in different ways *e.g.* 1) the irradiated males may be mated individually to virgin females or 2) mass matings for restricted periods could be used. In the choice between these two methods one has to consider the difficulty in the first method with regard to having an exact and common time table for all the males, and, besides this, the variation in the results between the mating periods could be too small to be detected. The second method might give an uncertain result as one does not know the copulation frequency, but it has the advantage that one will have a well defined length of time for the mating periods. The limits could be ± 1 min. For

this method commercial plastic boxes are used as population cages. This technique made it possible to study changes in mutation rate in successively younger germ cells at the time of irradiation.

The males were taken out when 0—1 day old and then irradiated when they awoke after etherization or were kept without females for 3 days prior to irradiation. The males were irradiated with a dose of 1100 r (2 min.) by a Skandia Intensiv apparatus operated at 170 kV, 15 mA and with an inherent filtration corresponding to 2 mm Al. Immediately after irradiation the males were transferred to a mating cage. The first mating period lasted from 0 to 4 hours a.i.* but from the 4th day and onwards every mating period consisted of 24 hours. Between the first mating period and the 4th day the irradiated males were kept with an excess of originally virgin females.

AUERBACH and MOSER (1953) have shown that the rate at which sperm is being utilized is sensitive to experimental conditions and the importance of using the right mating procedure is also pointed out by AUERBACH (1954). Therefore, in order to have a more secure shift in the sensitivity pattern, successive mating periods were used, each of 24 hours. Each mating period then represents successively younger germ cells at the time of irradiation. At the end of each mating period the males and females were separated. The males were transferred to a new mating cage and new virgin females were introduced. The old females were placed in vials, 5—7 in each. They were allowed to oviposit for three 3-day periods. The vials were emptied on the 12th and the 15th day and the offspring were recorded.

RESULTS

LÜNING (1952 a and b), proved that chromosome breakability varied very definitely within short intervals with the different stages of spermatogenesis. He used dominant lethals and hyperploid males as indication of genetical damage. SÄVHAGEN (1961) studied the frequency of induced XO males at different dose-levels in successive mating periods and, at the same time, induced autosomal crossing-over in the males scored on the 7th day after irradiation and onwards. She observed an increase in the yield of XO males from the 5th day after irradiation. The frequency reaches a peak during the 8th day after irradiation whereupon there is a drop towards the 10th day.

The purpose of the present experiment was to study the frequency

* Henceforth the abbreviation a.i. will be used for after irradiation.

TABLE 1. *Losses of paternal sex chromosomes and non-disjunction between the paternal X and Y chromosomes after irradiation of 0—1 day old males. *=heterogeneity within the material.*

Mating period after irradiation	XO ♂♂	Wild type ♀♀	Number of progeny tested wild type females due to		Total offspring	Per cent XO ♂♂	Per cent wild type ♀♀
			non-disj.	crossing-over			
0—4 hrs	94	2	1	—	20,538	0.46	0.01
4th day	205	20	6	—	62,692	0.33	0.03
5th day	377	14	8	—	50,552	0.75	0.03
6th day	328	2	2	—	24,735	1.33	0.01
7th day	400	40	26	1	18,100	2.21*	0.22*
8th day	463	68	44	2	13,350	3.47*	0.51
9th day	186	15	9	—	7,772	2.39*	0.19
10th day	152	49	49	—	27,946	0.54	0.18
11th day	137	42	29	—	30,044	0.46	0.14

of induced genetical damage and, at the same time, also to study the treated cell stages through a more direct method. As an indication of genetical damage the occurrence of XO males has been used, and, as a criterion of early meiosis (prior to anaphase I), the induction of non-disjunction between the X and Y chromosomes in the males. The results are presented in Tables 1—4. Each experiment consisted of several repeats. The heterogeneity was analysed by χ^2-test. Heterogeneity was only observed during the 7th—9th day after treatment of 0—1 day old males (Table 1). This is consistent with earlier findings (SÄVHAGEN, 1961).

From Table 1, which represents the results after irradiation of 0—1 day old males, it is seen that the amount of XO males rises steeply from the 5th day, reaches a peak on the 8th day after treatment and then decreases towards the 11th day (Fig. 3). This is in agreement with earlier findings (SÄVHAGEN, 1960, 1961). Since XO males are due to losses of the paternal X and/or Y-chromosomes both females and males are recorded and the rate of XO males per total offspring is calculated. Through χ^2-analysis it is established that the increase of XO males from 4th—8th day after treatment is statistically significant (4th vs 5th day and 5th vs 6th day=$P<0.0005$, 6th vs 7th day=$P<0.0005$; 7th vs 8th day=$P<0.0005$). The observed decrease of XO males is also significant (8th vs 9th day and 9th vs 10th day=$P<0.0005$).

If one examines the frequency of induced wild type females from

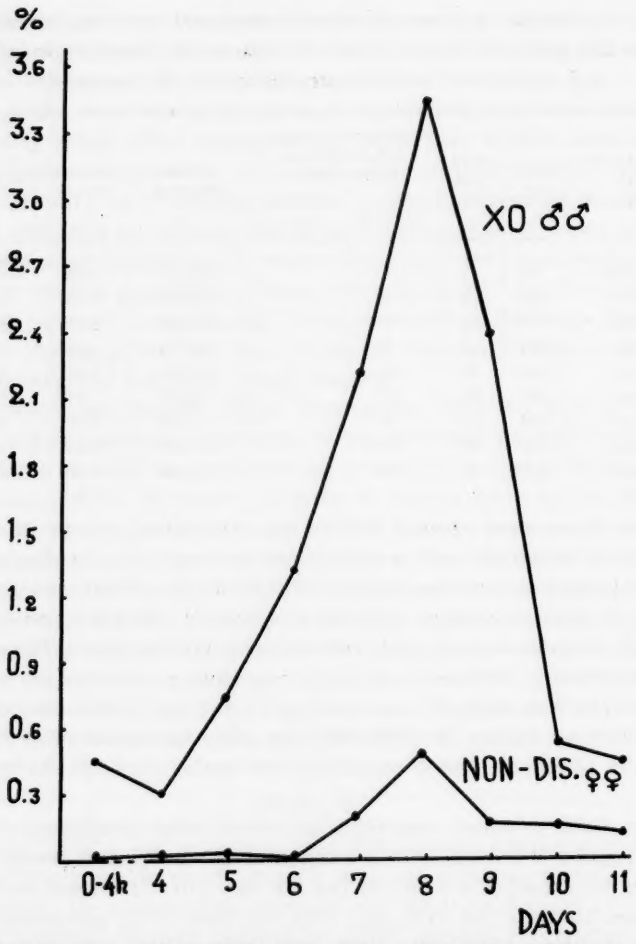

Fig. 3. The frequency of induced XO males and non-disjunction (wild type) females after irradiation of 0—1 day old males.

irradiated 0—1 day old males (Table 1, Fig. 3) one finds that the rate is practically unchanged from the first mating period until the 6th day after irradiation. The rate of induced non-disjunction females is at the same level as in the control series (Table 3). On the 7th day after irradiation there is a sharp increase in the frequency of induced wild type females. The frequency reaches a peak during the 8th day after irradia-

TABLE 2. *Losses of paternal sex-chromosomes and non-disjunction between the paternal X and Y chromosomes after irradiation of 3—4 day old males.*

Mating period after irradiation	XO ♂♂	Wild type ♀♀	Number of progeny tested wild type females due to		Total offspring	Per cent XO ♂♂	Per cent wild type ♀♀
			non-disj.	crossing-over			
0—4 hrs	142	15	8	—	44,635	0.32	0.03
4th day	84	16	15	—	43,016	0.20	0.04
5th day	168	7	4	—	38,766	0.43	0.02
6th day	121	6	3	—	17,818	0.68	0.03
7th day	176	17	12	—	14,155	1.24	0.12
8th day	181	25	17	—	11,706	1.55	0.21
9th day	84	11	7	—	8,724	0.96	0.13
10th day	41	12	6	—	8,312	0.49	0.14
11th day	15	4	4	—	7,807	0.19	0.05

tion, followed by a drop towards the 11th day. The rise in the frequency of obtained wild type females between the 6th—7th day and 7th—8th day as well as the decrease between the 8th—9th day after treatment is statistically significant ($P<0.0005$). As the wild type females also may arise through induced crossing-over between the X and Y chromosome, these females were tested individually. The majority of

TABLE 3. *Losses of paternal sex-chromosomes and non-disjunction between the paternal X and Y chromosomes in the control series.*

Mating periods	XO ♂♂	Wild type ♀♀	Number of progeny tested wild type females due to		Total offspring	Per cent XO ♂♂	Per cent wild type ♀♀
			non-disj.	crossing-over			
0—4 hrs	6	2	1	1	14,958	0.04	0.01
4th day	10	4	3	—	22,207	0.05	0.02
5th day	12	7	4	—	34,839	0.03	0.02
6th day	10	3	1	—	23,044	0.04	0.01
7th day	12	6	4	—	23,577	0.05	0.03
8th day	14	2	1	—	22,601	0.06	0.01
9th day	4	1	—	—	11,729	0.03	0.01
10th day	5	1	1	—	10,388	0.05	0.01
11th day	3	1	1	—	7,204	0.04	0.01
Total	76	27	16	1	170,547	0.045	0.016

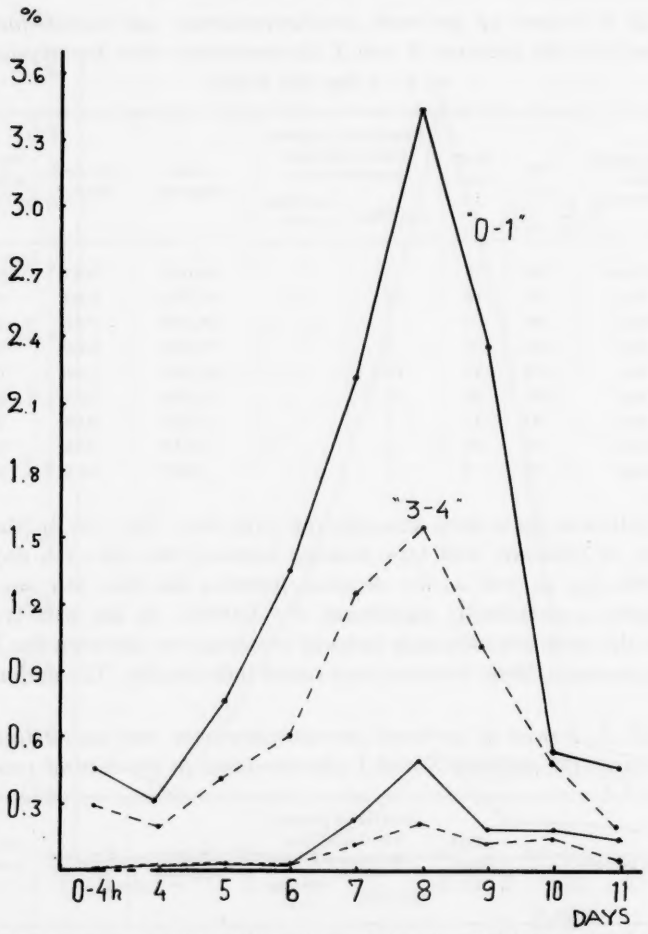

Fig. 4. The frequency of XO males and non-disjunction (wild type) females after irradiation of 0—1 and 3—4 day old males.

the wild type females were due to non-disjunction. It should be noted that in the first experiments no tests were performed and, besides this, it was not possible to test each female due to sterility and to the death of some females before the test was finished.

Of especial interest is to compare the frequency of induced XO males and the rate of induced non-disjunction females from the 4th to the

TABLE 4. *Per cent XO males and non-disjunction females after irradiation of 0—1 and 3—4 day old males.*

Mating period after irradiation	0—1 day old males		3—4 day old males	
	Per cent XO♂♂	Per cent non-disj.♀♀	Per cent XO♂♂	Per cent non-disj.♀♀
0—4 hrs	0.46	0.01	0.32	0.03
4th day	0.33	0.03	0.20	0.04
5th day	0.75	0.03	0.43	0.02
6th day	1.33	0.01	0.68	0.03
7th day	2.21	0.22	1.24	0.12
8th day	3.47	0.51	1.55	0.21
9th day	2.39	0.19	0.96	0.13
10th day	0.54	0.18	0.49	0.14
11th day	0.46	0.14	0.19	0.05

10th day a.i. (Fig. 3). It is seen that the peak of XO males coincides with the peak of non-disjunction females. Since the occurrence of non-disjunction females is used as a criterion of early meiosis (cells treated prior to anaphase I), there is an agreement between the highest X-ray sensitivity (measured through induced XO males) and cells treated at early meiotic stages.

The results after irradiation of 3—4 day old males are seen in Table 2. It is evident that the peaks of non-disjunction females and XO males will occur in the same mating periods as after irradiation of 0—1 day old males (Table 4, Fig. 4). Thus about the same sensitivity pattern is obtained after irradiation of 0—1 respectively 3—4 day old males (Fig. 4). There exists, however, a discrepancy in the amplitude of the sensitivity. The frequency of induced XO males as well as the rate of induced non-disjunction females is much lower in experiments with 3—4 day old males. This difference is statistically significant up to and including the 9th day for XO males and the 8th day for non-disjunction females. There is a fairly good agreement between these findings and LÜNING's (1952 c) conclusions that 6—7 day old males vary much less than 0—1 day old males between different stages of spermiogenesis.

On the basis of the present material the following conclusions may be drawn: 1) the frequency of induced XO males in the first mating period (0—4 hours a.i.; mature spermatozoa) versus the rate of induced XO males in the 7th—10th days after irradiation confirms LÜNING's findings that more damages are induced in the last mentioned period.

2) according to MAVOR's (1924) findings that X-rays applied to eggs during maturation causes non-disjunction one may conclude from the obtained results with wild type (non-disjunction) females, that the mating periods from the 7th—10th (11th) day a.i. correspond to cells which at the time of treatment were in an early meiosis (prior to anaphase I). 3) the peak of sensitivity (as measured by the frequency of induced XO males) coincides with the maximal yield of induced non-disjunction females, and hence, it might correspond to cells treated during early meiosis. 4) when comparing the curves for XO males with the curves for non-disjunction females one notices that the rise in the frequency of induced XO males has already started on the 5th day after irradiation, at a time, where there is no sign of induced non-disjunction. This indicates that the high breakability remains during a period after meiosis I. An observation which is confirmed by a study of induced translocations (SÄVHAGEN, 1960).

DISCUSSION

It is a well-known fact that the testes of adult *Drosophila* males contain germ cells in different developmental stages (COOPER, 1950). Several attempts have been made to translate the observed genetical effect to a pattern of sensitivity to irradiation of the testes. Different brood-techniques are used and the indication of induced genetic damage has varied from one experiment to another. As the main criteria for delimiting the spermatocyte and spermatogonial stages, the occurrence of a period of high sterility, first clear crossover and clusters of mutations are used.

In the present study the frequency of induced non-disjunction between the paternal X and Y chromosomes is used as the main criterion for cells treated during meiosis. From this experiment it is seen that the treated meiotic cells became available for insemination during the 7th—9th days after irradiation. In the same experiment the frequency of males, supposed to be XO males were studied. The occurrence of these males, henceforth called XO males, was taken as an indication of induced genetic damage. The peak in the rate of induced XO males was obtained in the mating periods from the 7th—9th days after irradiation. Thus one may conclude that the most sensitive stage must correspond to cells treated in early meiotic stage (prior to anaphase I).

When discussing the sensitivity pattern for induced genetic damage during various stages of spermio- and spermatogenesis it has to be pointed out that XO males do not represent a simple sign of induced

genetic damage. According to MULLER (1940) the XO males are thought to be the result of elimination of the X and/or Y chromosome caused by simple breaks or reunion of the broken ends to form dicentric and acentric chromosomes. From the present experiment it is evident that the XO males in addition to breakage are due to induced non-disjunction. Besides this there might of course exist other disturbances in the chromosome movements resulting in nullo-XY-sperms. Thus it is obvious that XO males might be the consequence of several different types of induced genetic damage and, hence, the occurrence of XO males might be used as a general measure of genetic damage. Another method to test the over all genetic damage has been to study the hatchability of eggs, usually referred to as the dominant lethal-technique. This has the disadvantage that the unhatched eggs have to be checked to determine whether or not they are fertilized (AUERBACH, 1954), a check that is very laborious.

From this point of view it must be of an especial interest to try to distinguish between the rate of XO males due to breaks and the amount due to induced non-disjunction. First, one has to consider if there are equal chances for obtaining nullo-XY-sperm and XY-sperm. Already GERSHENSON (1933) suggested that a chromosome which does not pair with its homologe might be lost during meiotic divisions. He made this suggestion in order to explain the discrepancy between exceptional males and females resulting from primary non-disjunction in the male. This problem has further been studied by SANDLER and BRAVER (1954) who concluded that the deficiency in the recovery of both the X and Y chromosome simultaneously is a function of the loss of one or both of these chromosomes during meiosis. They also made an estimate of the relative frequencies of losses. Their results showed that the Y chromosome in an XY-male was lost in a much higher degree than the X-chromosome. Finally, they suggest that the normal disjunction is dependent upon the pairing of heterochromatic regions.

Turning to the control series (Table 3) one finds that the frequency of spontaneously arising XO males does not differ statistically between the different mating periods, neither does the rate of non-disjunction females. The results may hence be pooled. A total offspring of 170,547 is obtained and among them 76 XO males and 27 non-disjunction females. Assuming that all the observed XO males and all wild type females in the control series (Table 3) are due to spontaneously occurring non-disjunction it seems legitimate to suppose that, also in the irradiated series, males and females occurring from induced non-dis-

junction, should be observed in the same ratio viz. 76:27=2.82. By multiplying the observed percentages of induced non-disjunction females in Tables 1 and 2 by 2.82 one would, hence, obtain a rough estimate of the amount of XO males due to non-disjunction. After reduction of this calculated percentage of XO males due to non-disjunction, the remaining XO males would be due to induced breakage and other disturbances giving rise to elimination of the X and Y chromosome. The results of these calculations are seen in Table 5.

From Table 5 it is seen that even after this reduction, the highest yield of XO males is obtained on the 8th day after irradiation, and that in no case a lower frequency of XO males is obtained during the 7th—9th days after irradiation than in the 5th—6th day after treatment. These results might indicate that the stage of highest breakability coincides with early meiosis. VON BORSTEL (1955) studying the response of meiotic stages in *Habrobracon* eggs to nitrogen mustard, observed that "the first meiotic metaphase is approximately 25 times as sensitive to nitrogen mustard as the first meiotic prophase". An observation which parallels results obtained after X-ray treatment (WHITING, 1945 a). Thus VON BORSTEL suggests that "the relative sensitivity of a given type of meiotic cells to mutagenic agents may be independent of the mutagen but dependent on the stage of the chromosome cycle".

The present results are contradictory to earlier suggestions concerning the sensitivity pattern of *Drosophila* male germ cells to different mutagens. LÜNING (1952 a) suggested that the most sensitive stage measured through induced dominant lethals (7—10 days a.i.) would correspond to maturing spermatids. AUERBACH (1954) using mating periods consisting of 3 days, by analogy suggested that the first brood (1st—3rd day a.i.) was derived from mature sperm, the second brood (4th—6th day a.i.) mainly from cells treated during spermiogenesis; the third brood (7th—9th day a.i.) from treated spermatocytes and the fourth brood (10th—12th day a.i.) stemmed from cells treated as spermatogonia. In all AUERBACH's experiments the peak of mutation frequency measured through induced recessive lethals occurred in the second brood, while the period of excessive sterility occurred in the third brood.

The period of excessive sterility and first occurrence of crossovers has been used by AUERBACH as criteria for cells treated in early meiosis, while bundles of identical and complementary crossover were used as an indication of treated spermatogonia. On account of these observations AUERBACH claims that "maximum sensitivity to the mutagenic

TABLE 5. *The calculated frequency of XO males due to breakage after irradiation of 0—1 and 3—4 day old males (1100 r).*

(For further explanation see the text)

	Mating period after irradiation	Per cent XO males after irradiation	Reduction of XO males due to non-disjunction*	Residual per cent XO males
0—1 day old males	4th day	0.33	0.09	0.24
	5th day	0.75	0.09	0.66
	6th day	1.33	0.03	1.30
	7th day	2.21	0.62	1.59
	8th day	3.47	1.44	2.03
	9th day	2.39	0.54	1.85
	10th day	0.54	0.51	0.03
	11th day	0.46	0.40	0.06
3—4 day old males	4th day	0.20	0.11	0.09
	5th day	0.43	0.06	0.37
	6th day	0.68	0.09	0.59
	7th day	1.24	0.34	0.90
	8th day	1.55	0.59	0.96
	9th day	0.96	0.37	0.59
	10th day	0.49	0.40	0.09
	11th day	0.19	0.14	0.05

*=per cent wild type females×2.82. For the factor 2.82 see the text.

effects of irradiation therefore occurs at a stage of spermatogenesis which is intermediate between early meiosis and the attainment of complete maturity by the sperm". That the period of excessive sterility corresponds to the rate at which early spermatocytes are destroyed (causing lack of sperm) was already suggested by FRIESEN (1937). The validity of this observation was confirmed by AUERBACH (1954).

Turning to the present material it is seen that the first sign of sterility (low fertility) is observed on the 6th day after treatment whereas "excessive sterility" is obtained on the 9th day after irradiation for 0—1 day old males and during the 9th—11th day for 3—4 day old males, which thus in part is in agreement with AUERBACH's observations. WELSHONS and RUSSEL (1957) on the other hand, found that both secondary spermatogonia and young spermatocytes are sensitive to the killing effect of X-rays and they suggested that the destruction of these sensitive cells results in a period of temporary sterility following irradiation of adult males.

AUERBACH's suggestion that the third brood (7th—9th day a.i.) corresponds to cells treated as spermatocytes is consistent with the results obtained in the present experiments. In AUERBACH's experiments, however, the peak of mutation frequency is obtained in the second brood (5th—7th day) and in the present investigation the highest yield of XO males is observed during the 7th—9th days after irradiation (early meiosis). Since the difference between the sensitivity pattern obtained in this investigation and that suggested by AUERBACH seems to be important, it is of interest to discuss the factors which may influence this cell stage/sensitivity relationship.

It was pointed out earlier that in studies of mutation rates it is necessary to consider both the age of the males at the time of irradiation and the mating periods in relation to the treatment. In AUERBACH's experiments the age of the males at treatment varied between 0—4 days and in the present experiment both 0—1 day and 3—4 day old males have been irradiated. Obviously the results from the present experiments show that for 0—4 day old males the age of the male at treatment does not influence the course of meiosis. Hence, the main difference between these two investigations is the brood technique, the stocks used, and the criteria of induced genetical damage.

Already AUERBACH (1954) pointed out that the method of sperm sampling with broods consisting of three days is too rough to allow a more accurate analysis of the changes in sensitivity. A shift of the mutation peak may be caused by a difference in the breeding procedure as suggested by KHISHIN (1955), who also supposed that a shift may be caused by the use of insufficient number of females per males. Thus it seems possible that the difference in brood technique may influence the results, but it seems also probable that the criteria of induced genetic damage may play a very important role. This is further strengthened by IVES' findings (1960). He also observed that the peak in the rate of induced sex-linked recessive lethals occurred during the 5th and 6th day after treatment, in spite of the fact that he has used a day by day brood technique. This idea is consistent with BATEMAN and CHANDLEY's results (1959). That the stocks used is of importance has been demonstrated by STRØMNÆS (1959).

FAHMY and FAHMY (1954, 1955) have used a combined cytological and genetical technique in order to determine the sensitivity pattern to a chemical mutagen. On account of this method and further experiments (1957), they have suggested "that sperm ejaculated in the first four matings (4 broods: 12 days after treatment) has been derived from

post-meiotic germ cells (sperm and spermatids), whereas sperm utilized in later matings (5th brood onwards: more than 12 days after treatment) has been derived from meiotic and premeiotic germ cells (spermatocytes and spermatogonia)". As indication of genetical damage they have used the yield of dominant and recessive lethals. The fact that they obtain the highest yield of dominant lethals in the first brood (1—3 day after treatment) indicates according to FAHMY and FAHMY (1954), that mature sperm is the most sensitive stage under the effect of imine. They also point out that this constitutes a difference between chemical and X-ray induced damages. For sex-linked recessive lethals they (1955) concluded that "Maximal mutation occurs among sperm utilized towards the end of the third brood (8—9 days after treatment) which is about the time spermatids at the very beginning of nuclear condensation are expected to be called upon for the supply of sperm". It is difficult to understand how FAHMY and FAHMY have been able through this technique to tell which type of affected (or apparently unaffected) cells will be used as mature sperm in the coming 4th—7th broods. The method used by FAHMY and FAHMY has recently been analysed and criticized by AUERBACH (1957, 1958 a).

There is always a danger to compare experiments when different mutagenic agents have been used. In the present experiment X-rays, and in FAHMY and FAHMY's investigations chemical mutagens have been used. These authors have also pointed out that the mutagenic pattern in the chromosomes are quite different for chemical mutagens compared with that for X-rays (1958). AUERBACH (1958 b), comparing the effects of radiomimetic chemicals and radiation, calls attention to the fact that there exists similarities, as well as differences, between mutations and chromosome breaks which have been produced by radiation or chemicals. "The most interesting differences is the greater degree of specificity which some chemical mutagens show in their effects on chromosome regions and individual genes."

Among the observations that all germ cells do not respond equally to irradiation and chemical mutagens there are in addition to experiments on adult *Drosophila melanogaster* males several investigations with the same purpose made on different developmental phases of the young animals (ALEXANDER, 1954, 1958; ALEXANDER and STONE, 1955; KHISHIN, 1955; OSTER, 1955, 1958; FRITZ-NIGGLI, 1958, 1959 a, b). KHISHIN (1955) claims that a more direct method of correlating mutation rates with the types of irradiated germ cells consists of treating successively younger developmental phases on the life cycle of the animal. He irra-

diated larvae, prepupae, pupae and adult flies. By analogy inference he obtained a sensitivity pattern of testis to irradiation, similar to that established by AUERBACH. He concludes that: "Mutation rate is low in all premeiotic stages up to and including growing primary spermatocytes which are preparing for divisions. It increases suddenly and spectaculary with the onset of meiosis, remains stationary during the meiotic divisions, and subsequently increases again up to a peak in the late spermatid stage, when transformation takes place into morphologically, although not yet physiologically mature sperm ('maturing spermatozoa'). Finally there is a drop in mutation rate, which in maturing and mature spermatozoa is considerably lower than at meiosis."

Against this method one can argue that there might exist big environmental differences between the cells in these developmental stages. KHISHIN has tried to check this through obtaining brood patterns for several of the treated developmental phases. Besides this KHISHIN has not been able to use the same X-ray dose for all stages. This must be a great disadvantage when one is going to compare the mutagenic rate in different stages of spermato- and spermiogenesis especially since MULLER *et al.* (1954) were not able to obtain linearity for irradiated germ cells delivered 7—9½ day after treatment. OSTER (1958), also irradiating different developmental stages of *Drosophila*, deduced the following order of decreasing sensitivity to X-rays: 1) spermatids and early cleavage stages, 2) spermatozoa in the female, 3) spermatozoa released one day after treatment, spermatozoa released two days after treatment and 4) spermatogonia. The idea that spermatids show the highest radiosensitivity is supported by SOBELS (1955, 1958).

That the results obtained by AUERBACH, KHISHIN, OSTER and SOBELS are in agreement might be explained by the use of sex-linked recessive lethals as indication of induced genetic damage. This idea is further strengthened when a comparison is made between the present results and that obtained by BATEMAN (1957). He has used the occurrence of hyperploid females as indication of induced genetic damage. After treatment of the *Drosophila* males with 1000 r BATEMAN obtained a continuous rise in the frequency of hyperploid females from the first to the 7th day, followed by a sharp decrease on the 9th day a.i. These data are in full agreement with the present result with XO males. However BATEMAN by analogy concluded that the drop on the 9th day a.i., corresponded to the intervention of meiosis and thus he claimed that the highest sensitivity (increased breakability) was found in spermatids.

In the meantime other investigators have attacked this problem.

FRITZ-NIGGLI (1958, 1959a, b) has treated about the same developmental stages as KHISHIN (1955). As indication of irradiation damages FRITZ-NIGGLI (1958) used dominant lethals. The highest frequency of dominant lethals is found 7 to 8 days after irradiation. By analogy inference she suggests that the 1st—4th day after treatment correspond to mature sperm, the 4th—7th day to spermatids, the 7th—10th day to meiosis and from the 10th day onwards to cells treated as spermatogonia. FRITZ-NIGGLI does not furnish any real proof of her suggestion that the broods from the 7th—10th days correspond to meiosis. But as far as is seen from her results, they are in agreement with the present findings.

IVES (1960) using a day-by-day mating technique has studied the rate of induced dominant visibles, hemizygous sex-linked mutations, number of offspring produced and induced crossovers in *Drosophila* males after treatment with cobalt 60 γ. By analogy mainly based on crossover data he suggests "that the mutation frequency reaches its highest level in sperm irradiated in the meiotic stages of spermatogenesis, clearly at a different stage from that at which the strongest adverse radiation effect on fecundity occurs". He also supposes, according to the observed crossover data, that sperm available for insemination during the 7th day have been treated as primary spermatocytes. In all experiments IVES observed a low fecundity on the 9th day followed by a recovery on the 10th or the 11th day. At the same time the observed rate of induced sex-linked recessive lethals has decreased to a comparatively low level, where it remains. IVES suggests that the 10-day sperm may have been irradiated in the mitotic stages between gonial and meiotic stages. On account of IVES' observations it would be of interest to try to compare the relation between fecundity and observed frequency of induced XO males and non-disjunction females in the present investigation. The observed correlation between the increase in fecundity and decrease in the rate of XO males and non-disjunction females parallels the observation made by IVES. This correlation is obvious when comparing the effect after irradiation of 0—1 day old males. For 3—4 day old males, however, there is no sign of recovery of fecundity on the 11th day, while the rate of XO males and wild type females has decreased.

OFTEDAL (1959) has tried to measure the time needed for spermatogenesis by means of tagging sperm with P^{32}. He claims that "the period between the last DNA-synthesis in spermatocytes and the transfer of mature sperm derived from these cells, at the most is eight days and probably not shorter than six, as indicated by P^{32} incorporation". OFTEDAL's observations that, "P^{32} incorporated into the DNA of mature sperm

is recovered seven or eight days after a single meal, under conditions of excessive mating, which coincides with maximum genetic effect", are interesting, since HOWARD and PELC (1952) reported that, "in *Vicia* the stages of sensitivity to chemical and radiation breakage coincides neither with one another nor with time of DNA synthesis judged by P^{32} uptake". GAULDEN (1956), also points out that the stages of mitosis most sensitive to X-rays, are not involved in synthesis of DNA.

From the material in the present investigation it is suggested that the peak of sensitivity corresponds to cells treated during early meiosis. This is consistent with TAZIMA and ONIMARU's (1957) findings that early spermatocyte is an extraordinarily sensitive stage in the silkworm, and MOHR's (1919) observation that the youngest spermatocytes in the testis of grasshopper *Decticus*, are the stage most sensitive to irradiation. RAY (1957), studying X-ray sensitivity of meiotic stages of *Mormoniella* eggs observed a higher sensitivity of the first meiotic metaphase. SPARROW (1947, 1948, 1951) working with *Trillium*, showed a gradual increase of sensitivity from early meiotic prophase up to metaphase I. These findings coincide with observations made by WHITING (1945 a, b, 1949, 1955) and VON BORSTEL (1955) on *Habrobracon*, and by BOZEMAN and METZ (1949) on *Sciara*. HAQUE (1952) studying meiosis in *Tradescantia*, found that the stages of higher sensitivity are stages where the chromosomes are more condensed, whereas, the diffuse stages have a lower sensitivity.

There are numerous hypotheses proposed in order to explain the high sensitivity of chromosomes treated during metaphase I. SPARROW (1944, 1947) suggested that the high sensitivity to X-rays may be correlated with nucleic acid metabolism since high and low sensitivity stages correspond to stages of high and low concentrations of DNA in the chromosomes. HOWARD and PELC (1952) confirm that DNA synthesis is radiosensitive. BOZEMAN and METZ (1949) claim that the breakdown of the nuclear membrane is of importance. SPARROW and MALDAWER (1950) conclude that rejoinability is low during meiosis. WHITING (1945 a, b) proposed that tension on the chromosomes is greater in the sensitive stage and that this tension might influence restitution.

The existence of disturbances in the chromosomal movement towards the poles after irradiation was observed by BARRATT and ARNOLD (1911) and ALBERTO and POLITZER (1923). Difficulties for chromosomes to separate in anaphase I was found by WHITE (1937), who studied the effect of irradiation on meiosis of *Orthoptera*. He also observed that

cells in metaphase I were completely destroyed, while the chromosomes are not affected during prophase. WHITE suggested that the nuclear membrane acts in a protective way, isolating the chromosomes from substances which are present in the cytoplasm of irradiated cells. Induced stickiness between the chromosomes is also observed by SAX and SWANSON (1941) who describe the clumping and sticking together of metaphase chromosomes in *Tradescantia*. This is confirmed by RILEY (1936) and FRITZ-NIGGLI (1956). That stickiness might affect the rate of non-disjunction was pointed out by RAPOPORT (1938). v. GELEI and CSIK (1940) studying non-disjunction in *Drosophila melanogaster* females showed that environmental factors may influence the process of disjunction. Thus it seems generally accepted that the stages in the nuclear cycle are of importance in determining the degee of sensitivity to X-rays.

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SUMMARY

(1) The present paper represents an attempt to obtain additional information on the course of meiosis, or, when sperm, which at the time of treatment were in meiosis, are ejaculated. At the same time an attempt is made to correlate this information with a sensitivity pattern. By use of a dual-purpose stock it was possible to study the frequency of induced XO males and induced non-disjunction between the paternal X and Y chromosomes.

(2) 0—1 and 3—4 day old y^{1e}; sc^{8}Y males were irradiated (1100 r) and mated to virgin *y w sn* females in successive mating periods. The first mating period was, according to LÜNING's findings, delimited to 0—4 hours after irradiation but from the 4th day and on each mating period consisted of 24 hours.

(3) It is shown that irradiation of *Drosophila* males causes non-disjunction between the paternal X and Y chromosomes. Hence, the occurrence of an increase in the frequency of non-disjunction in sperm ejaculated during a limited period after irradiation must be taken as an indication that those spermatozoa were in meiosis at the time of treatment.

(4) The observed frequency of induced XO males rises steeply from the 5th day, reaches a peak on the 8th day after irradiation and then decreases towards the 11th day.

(5) The rate of induced wild type females (non-disjunction females) is practically unchanged from the first mating period (from 0 to 4 hours a.i.) until the 6th day a.i. On the 7th day there is a sharp increase in the frequency of induced wild type females. The frequency reaches a peak during the 8th day followed by a drop towards the 11th day after irradiation.

(6) When comparing the frequency of induced XO males and the rate of induced non-disjunction females it is seen that the peak of XO males coincides with the peak of non-disjunction females. Since the occurrence of non-disjunction females is used as a criterion of early meiosis (cells treated prior to anaphase I), there is an agreement between the highest X-ray sensitivity (measured through induced XO males) and cells treated at early meiosis.

(7) About the same sensitivity pattern is obtained after irradiation of 0—1 and 3—4 day old males. However, the frequency of XO males as well as the rate of induced non-disjunction females is much lower in experiments with 3—4 day old males. Thus, in agreement with LÜNING's observations (1952 c) for 6—7 day old males, 3—4 day old males have a lower variation during various stages of spermiogenesis.

(8) When comparing the curves for XO males with the curves for non-disjunction females it is observed that the rise in the frequency of induced XO males has already started on the 5th day a.i., at a time where there is no sign of induced non-disjunction. This indicates that the high breakability remains during a period after meiosis I. An observation which is confirmed by a study of induced translocations (SÄVHAGEN, 1960).

(9) On the basis of the difference between the sensitivity pattern obtained in this investigation and that suggested by AUERBACH (1954), factors which may influence this cell stage/sensitivity relationship are discussed.

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REPEATED ENDOREDUPLICATION IN A MOUSE CELL

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AS material for our investigations of the normal mouse karyotype (LEVAN, HSU and STICH, unpublished), we used primary cultures of cells from individual mouse embryos. Mitotic aberrations were noticed now and then, including structural and numeric variations. Sometimes specific types of chromosome deviations were characteristic of a mouse strain or of an individual mouse embryo. Since cell suspensions derived from entire embryos were cultivated, differences between embryos may involve differences among the tissues grown in each culture. Structural chromosome aberrations were undoubtedly more frequent in certain mouse strains, while some embryos seemed to have a higher frequency of chromosome doublings than others.

The chromosome doubling mechanism named endoreduplication, or ER, by LEVAN and HAUSCHKA (1953) was often observed, and it was especially frequent in the ten embryos of Swiss mice studied. In one female Swiss embryo a beautiful case of ER was noted, repeated twice in the same mitotic cycle of a cell. Since only few cases of repeated ER have been described, a report of the present case seems appropriate.

The general appearance of the cell is shown in Fig. 1, while details of the cell are represented in Fig. 2. The total chromosome number is around 320. The arrangement of the chromosomes is characteristic: It is beyond doubt that they are arranged in bundles of eight, although the bundles have become partially broken up. The squashing must necessarily have acted upsetting on the original arrangement, and it is possible that other factors too have been of influence. That each original bundle contains eight chromosomes (16 chromatids) is clear from bundles in which the chromosomes exhibit a marker quality. This is the case with the eight chromosomes with proximal heterochromatic

knobs at the arrow in Fig. 1, and separately drawn in Fig. 2 d. The eightfold repetition of each single chromosome gives a rare opportunity to distinguish between significant and more incidental chromosome traits. If a certain feature, a constriction, a gradient etc., is found in all 16 chromatids of a bundle, it is safe to conclude that it is a basic characteristic of that chromosome.

The unit within each bundle is the chromosome, *i.e.* two chromatids joined at the centromeric region. The chromosomes have the tuning fork appearance typical of colchicine-treated mouse chromosomes. Within the least disturbed bundles, the centromeres are still close together, indicating that this part is the last to fall apart after the chromosome reproduction. In the broken-up bundles an interesting hierarchy may be discerned in the separation of chromosomes. Thus, quartets have often been removed from each other, and when quartets fall apart, still pairs of chromosomes stay together. This is interesting and may imply that some kind of connection between the centromeres actually remains after their division, the intimateness of this connection being a function of the time elapsed after the reproduction of the centromere. Other causes of this phenomenon may be thought of, as the possible occurrence, during the preceding interphase, of more than one endomitotic contraction cycle leading to separation and partial drifting apart of the endoreduplicated chromosomes. That most of them still remain together in bundles may be due to the development of lobes in the interphase nucleus confining individual bundles to separate compartments. The general distribution of the chromosomes in Fig. 1 actually suggests a lobated nucleus in the preceding resting stage. Unfortunately, a single case as the present does not permit any definite conclusions as to the causes of the interesting hierarchic grouping of the endoreduplicated chromosomes. The possibility of experimentally inducing ER would be extremely helpful for the study of this problem. Since it is a common observation that the frequency of ER is highly variable even in cultures of the same material, it seems that environmental factors favouring ER might be found.

The ER mitosis eventually leads to the auto-orientation of each single chromosome and the separation of the chromatids into two daughter nuclei (TJIO and LEVAN, 1954, Fig. 12 f). Thus, the present cell would have formed daughter cells with 320 chromosomes each, and the considerable step from diploidy to 16-ploidy would have been taken during one mitotic cycle by the fourfold reproduction of each chromosome.

In the paper by LEVAN and HAUSCHKA quoted, one cell was pictured

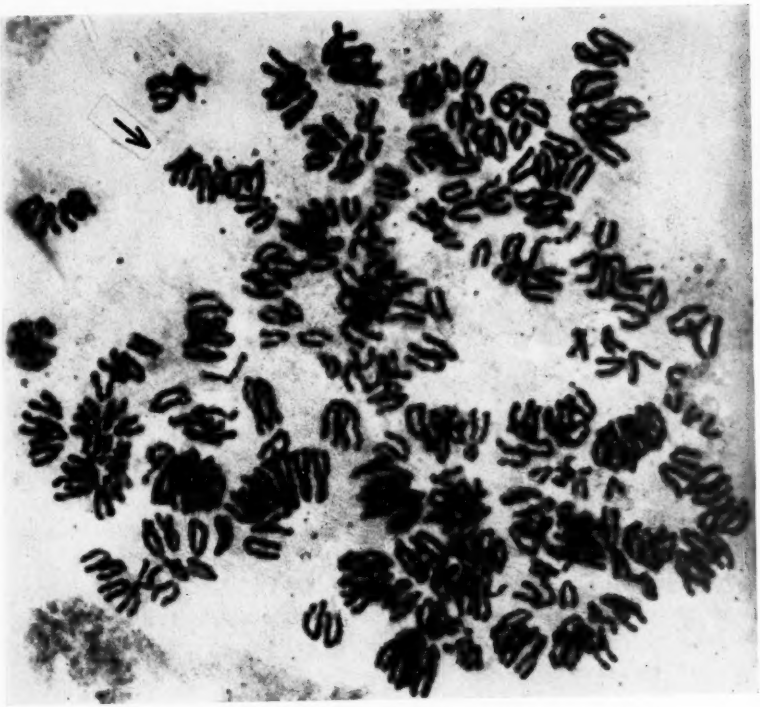

Fig. 1. General appearance of endoreduplicated cell. — × 1400.

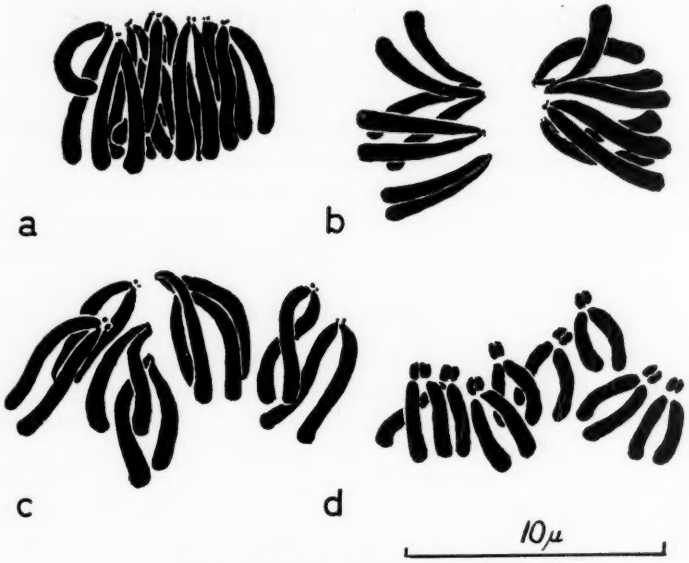

Fig. 2. Details from Fig. 1. — × 4000.

from the 15091a carcinoma with quartets of chromosomes (*l.c.*, Fig. 7 H, I), whereas one cell from the Krebs-2 carcinoma with a remarkably high chromosome number (more than 1000 chromosomes) showed aggregates of perhaps as many as 32 single chromosomes, indicating a sixfold reproduction during the preceding interphase (*l.c.*, Fig. 8 F—H). The clarity of chromosome structure in the present case makes the underlying chromosomal mechanism more perspicuous than in the latter of the two reported cases of repeated ER. The former of them was interesting because quartets of chromosomes were still firmly joined at their centromeres.

Acknowledgement. — Financial support by grant No. G-16241 from the National Science Foundation is gratefully acknowledged.

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BREEDING PATTERNS OF A NORTH SWEDISH PARISH*

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I. INTRODUCTION

STUDIES of the population structure in man are important in understanding the exchange of genetic material between different human groups. Researches on demographic factors relevant for population genetics are often termed genetic demography.

The term isolate goes back to the Swedish scientist S. WAHLUND (1928). Wahlund pointed out that large human populations are composed of smaller population units isolated from each other by various factors. Thus we may find geographical, social or religious isolates. Within the isolate most individuals will find their marriage partner. The size of an isolate, that is the numbers of inhabitants within the isolated group, may be measured in different ways. DAHLBERG (1929) pointed out that the frequency of consanguineous marriages was closely related to the size of the isolate. DAHLBERG (1943) has also emphazised the importance of the process called "the breaking up of isolates", that is the continuous decrease of marriages within small isolated areas. This process has been possible to follow in many countries. Thus SUTTER and TABAH (1948) have demonstrated a clear decrease of consanguineous marriages in all departments (administrative divisions) of France.

FREIRE-MAIA (1957), comparing the inbreeding levels in different countries, found the highest local rates of inbreeding in Brazil, Japan, India and Israel. In most European countries the frequency of first cousin marriages generally is less than 1 per cent. In this connection it must be remembered, however, that the figures from different countries are not strictly comparable. In Roman Catholic countries, where consanguineous marriages are registered in the church books, collections of data on the frequency of e.g. cousin marriages are not so laborious

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as in Sweden, where no such notes are available. Therefore, in Sweden, investigations concerning cousin marriages are restricted to a few areas, and all these studies have been made using the genealogical method starting from the parish books.

FRACCARO (1958) has summarized data on the frequency of first cousin marriages from six rural communities in Sweden. BÖÖK (1948) has studied three parishes in the county of Norrbotten. In the parish of Muonionalusta he obtained a frequency of 6.81 per cent first cousin marriages, while the neighbouring parishes Junosuando and Pajala had 2.85 and 0.95 per cent respectively. In the parish of Ovanåker (county of Gävleborg) FRACCARO (1958) found 0.57 per cent first cousin marriages. BÖÖK and MÅWE (1955) observed in the parish of Östmark (county of Värmland) an incidence of cousin marriages that was 1.23 per cent for the period 1905—1925 and 1.31 per cent for the period 1910—1941. The southwest Swedish islands of Orust and Tjörn (LARSSON and SJÖGREN, 1954) have relatively high incidences (2.35 and 3.09 per cent respectively). Finally in Börringe (LARSON, 1956) in the southernmost county of Sweden (the county of Malmöhus) the frequency is 1.74 per cent. FRACCARO (1958) points out that the relatively high frequency of cousin marriages in Börringe, a community with no geographical isolation, high population density and mobility, indicates that other factors than geographical isolation may be important. ALSTRÖM (1958) has made an extensive study of first cousin marriages in different parts of Sweden for the period 1750—1844. He found certain geographical variations of the incidence of cousin marriages. These incidences were fairly constant in different periods of time.

Reproductive isolation may be studied also by more direct methods than consanguinity rates. CAVALLI-SFORZA (1959) has used "endogamy", that is the percentage of marriages in which both partners reside in the same parish, as a measurement of the mobility of populations. In the Parma diocese of northern Italy the percentage of endogamy varied between 34 and 77 per cent with a mean of 54.5 per cent. BECKMAN (1959) studying the same phenomenon in six counties of northern Sweden (then called "regional intramarriage") found essentially the same figures (a range between 31 and 77 per cent). Endogamy (regional intramarriage) is strongly dependent on population density and size of the area.

The aim of this study is to compare data on consanguineous marriages with direct estimates of the geographical isolation of the same areas.

Fig. 1. Location of the parish of Offerdal (black area).
Hatched area: the county of Jämtland.

II. THE PRESENT INVESTIGATION

1. The investigation area

The parish of Offerdal which is located in the western part of the county of Jämtland (see Fig. 1) has very good church archives, a list of which was published in 1910 by the late vicar F. WIMMERCRANTZ. Further the genealogy of the parish is thoroughly studied by a local genealogist, KARL E. NYQVIST, whose eminent knowledge of the parish population aquired through lifelong genealogical studies has been of great importance when carrying out this study.

The area comprises 1,975 square kilometers. The western part is a mountainous area bordering on Norway, which is sparsely inhabited. The parish has an elongated shape along a water path and in the centre there is a lake (see Fig. 2). In the south and the north there are mountain areas isolating the parish from neighbouring parishes. In other words we deal with something like a valley-population. In 1960 the number of inhabitants was 3,946 and the population number is steadily decreasing. The northwestern part of the parish is practically uninhabited and the greatest population density is in the southeast, where the main agriculture area is located.

There are about 45 villages with a mean population size of 80—90 individuals. The locations of the villages are shown in Fig. 2. It is possible to make a rather natural division (from a physico-geographical

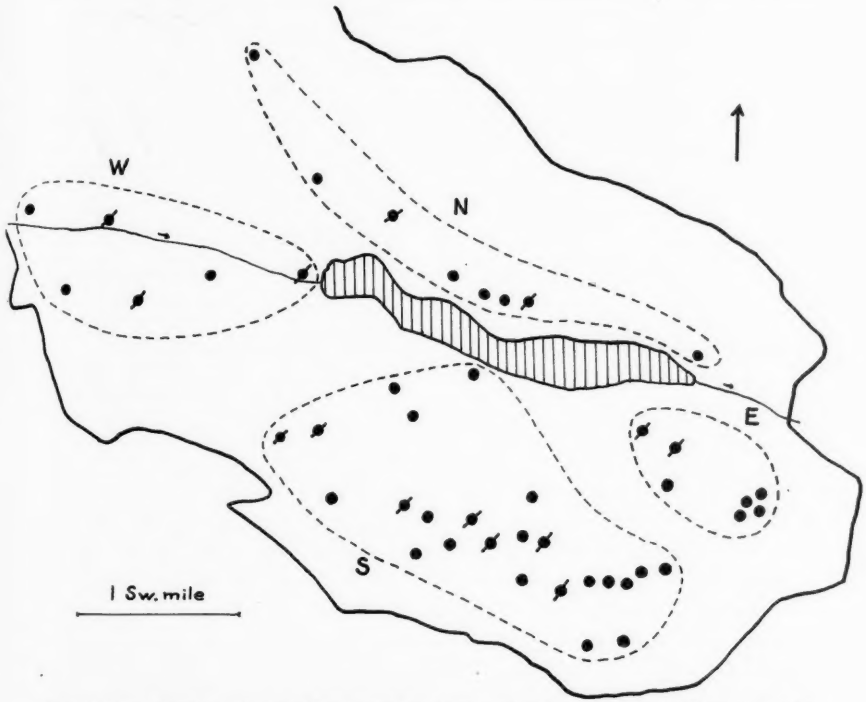

Fig. 2. Map showing the inhabited, southeastern part of the parish of Offerdal. Black dots indicate villages and dots crossed by a line villages with occurrence of cousin marriages. The four different groups of villages (N, E, S and W) are surrounded by hatched lines. In the middle of the parish there is a lake.

point of view) of the villages into four groups: One area south of the lake, one north of the lake, one in the west mountainous area and finally a fourth area in the east. These areas are indicated in Fig. 2 with hatched lines and the symbols S, N, W, and E. The southern area is the one that was first colonized and has the greatest population number and population density. This area has also the best communications. The western and northern areas are not agricultural areas in the same sense as the southern area, and here the population has some Lappish admixture. The village in the extreme northwest of the northern area is a pure Lapp village. From a physico-geographical point of view the eastern area is not very strongly divided from the other areas. The population totals of the northern, western and eastern areas are of

TABLE 1. *Mean distance between partners in four different periods.*

Period	Distance between partners, km		Both from same village, %	No. of marriages
	Mean and mean error	Standard dev.		
1861—1875	6.11±0.42	6.79	37.60	258
1876—1890	6.05±0.41	6.87	31.77	277
1891—1905	5.97±0.48	7.70	39.15	258
1906—1920	6.60±0.49	8.86	41.61	310

about the same size, each of about 10—12 per cent of the whole population, while the southern area contains the greatest amount of the parish population.

2. Breeding pattern

The total number of marriages contracted during the period 1860—1920, with both partners residing in the parish, were examined. For all individuals in the 1,103 marriages thus obtained the place of residence (village) was recorded. The distance (in km:s) between the partners was measured on the map. The mean distance between the place of residence of the partners was fairly constant (around 6 km:s) for four consecutive 15 year periods, and the increase shown in the latest period is not significant (Table 1). One would have expected a more pronounced increase of the distance between marriage partners in this period (1860—1920) since it is known to be characterized by a general increase of mobility, improvement of communications etc.

The frequency of marriages where both partners are from the same village varies between about 30 and 40 per cent. One would have expected a continuous decrease of this percentage. Instead there is a significant increase ($0.02 > P > 0.01$) of marriages within villages in the two latest periods (1891—1920) compared to the period 1876—1890.

Table 2 shows the intermarriage relations between the four subregions of the parish (cf. Fig. 2). In the table are also given the expected frequencies assuming random mating within the parish, that is, no effect of the geographical variation of residence of the marriage partners. The fact that marriages with partners from outside the parish are omitted will, of course, introduce a bias, and the calculated figures are then approximative.

Fig. 3 shows schematically the frequencies of marriages with females

TABLE 2. *Frequencies of marriages between partners from the four different subregions.*

The location of the subregions are found on map 2. Exp.=expected frequency assuming random mating within the parish.

Husband from subregion		Wife from subregion				Total
		N	E	S	W	
N	obs.	101	10	44	11	166
	exp.	22	18	108	18	
E	obs.	7	73	48	—	128
	exp.	17	14	83	14	
S	obs.	20	37	606	35	698
	exp.	91	77	453	77	
W	obs.	15	2	18	76	111
	exp.	15	12	72	12	
Total		143	122	716	122	1103

from different subregions when the male partner is from a certain subregion. We may note that men from the northern, western and southern subregions marry females from the two neighbouring subregions in about the same frequency, while in the eastern region there is a tendency for males to marry females from the southern region more often than from the northern region. Men from the northern subregion have also rather often married females from south, but males from south have relatively seldom married a female from the northern subregion. This "asymmetry" in the marriage pattern is most likely caused by both demographical and socio-economical factors such as excess of males in certain newly colonized areas and a tendency to avoid marriages which involve movement to poor areas.

If the observed and theoretically expected frequencies of marriages within the four regions are compared we will find that the ratio $\frac{\text{observed}}{\text{expected}}$ for the southern, northern, eastern and western areas are 1.3, 4.6, 5.2 and 6.3, respectively.

3. First cousin marriages

A survey of all existing marriages in the parish in 1960 was made. The frequency of first cousin marriages in the total parish is relatively high (see Table 3). Within the parish, however, there are significant

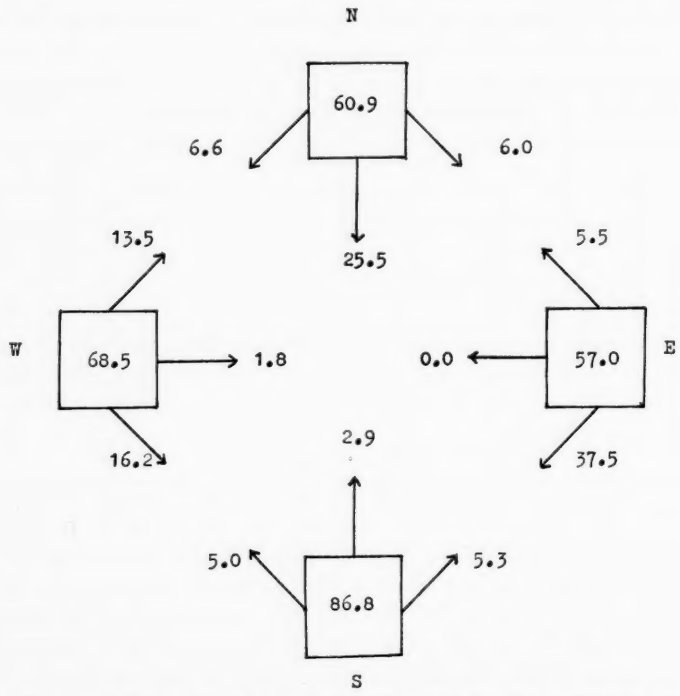

Fig. 3. Diagram showing, for the four different subregions, the percentage of men marrying women from the same or from neighbouring areas.

variations. Thus, in the western region there is as much as 5.5 per cent first cousin marriages. In the southern subregion, which is characterized by rather good communications, the percentage approaches rather "nor-

TABLE 3. *Incidence of first cousin marriages in the four different subregions.*

Subregion	No. of marriages	First cousin marriages	
		No.	%
N	143	4	2.80
E	92	2	2.17
S	706	8	1.13
W	108	6	5.56
Total	1049	20	1.91

mal" values. A comparison of the southern subregion to the three other ones shows that there is a significant difference in respect to the incidence of first cousin marriages ($\chi^2=6.87$, 1. d.f., $0.01>P>0.005$).

Villages containing cousin marriages are indicated in Fig. 2. There is a tendency to concentration in certain villages e.g. in one village 4 out of 39 marriages were cousin marriages.

III. DISCUSSION

It is notable that no distinct differences in the distance between marriage partners in different periods of time can be traced. The period selected for this investigation is known to be characterized by very pronounced changes on the socio-economical level among others the introduction of the timber industry.

The increase of the incidence of marriages within villages during the investigation period as well as the high presentday incidence of first cousin marriages indicate that the parish of Offerdal still is to be considered a rather isolated population. The breeding patterns of this population does not appear to have been essentially changed by the introduction of industrialism and the development of new means of communication.

It is interesting to compare the ratio $\frac{\text{observed}}{\text{expected}}$ frequencies of marriages within the four subregions with data on consanguineous marriages. In the southern area with 1.13 per cent cousin marriages this ratio is 1.3 and in the western area with 5.56 per cent cousin marriages the ratio is 6.3. The two other areas with a cousin marriage frequency between 2 and 3 per cent have intermediate values of the ratio. The frequency of marriages with both partners from the same area is strongly dependent on the size of the area and the population density. The areas and population densities of the present four subregions vary considerably and thus in this case the frequencies of "endogamy" are not directly comparable. The ratio used in this study thus evidently provides a better measurement of the regional isolation than does the per cent "endogamy".

It is remarkable that different subregions within such a comparatively small area as a Swedish parish may be so strongly isolated from each other. Thus, between the eastern and western areas in the parish of Offerdal there has been only two marriages during a period of sixty years. This means that the direct exchange of genetic material between those two subregions must have been extremely small.

SUMMARY

The breeding patterns of the north Swedish parish of Offerdal has been studied. During the period 1860—1920 the distance between the place of residence of marriage partners was rather constant (about 6 km:s). The incidence of marriages with both partners from the same village varies between 30—40 per cent in different periods of time, and has increased in the later periods. The frequency of first cousin marriages is relatively high and varies significantly within the parish. There seems to be an agreement between consanguinity data and direct estimates of the regional isolation.

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CYTOTAXONOMICAL STUDIES ON *VICIA CRACCA* L. AND *V. TENUIFOLIA* ROTH

I. CHROMOSOME NUMBERS AND KARYOTYPE EVOLUTION

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I. INTRODUCTION

VICIA CRACCA L. and *V. tenuifolia* ROTH are two closely related species of the section *Cracca* RIV. Sometimes (*e.g.* HEGI) the latter species is considered as a subspecies and called *V. cracca* subsp. *tenuifolia* (ROTH) GAUDIN. In addition there are two other subspecies, *V. cracca* subsp. *vulgaris* GAUDIN and *V. cracca* subsp. *Gerardi* (ALL.) GAUDIN. The two last named subspecies are the ones usually included in *V. cracca* L. In a critical taxonomical study, KOSTRAKIEWICZ (1951) found a number of good morphological differences between *V. cracca* L. and *V. tenuifolia* ROTH. According to KOSTRAKIEWICZ the two species ought to be kept separate. This is also done in the present paper.

The distribution area of *Vicia cracca* L. is very wide, comprising the whole of Europa and a large part of Asia as far as Sakhalin and Japan. It is one of the plants greatly favoured by man's action, growing mostly in meadows, fields, roadsides and other disturbed habitats. In addition it often occurs as an original member of the vegetation of gravelly or sandy seashores, cliffs, certain types of deciduous forests etc. It has been introduced to North America, Australia and New Zealand. GLEASON (1952), however, in his flora considered it as native in the north-eastern United States and adjacent Canada. BRITTON and BROWN (1913) in their original flora of that same area also considered it as native. Most American authors, however, regard it as introduced from Europe.

Vicia tenuifolia ROTH is more restricted in its distribution area. It occurs in Europe except for its northern parts and Britain, being more common than *V. cracca* in the Mediterranean and reaching into north-

western Africa. In Asia its distribution area extends from Lebanon to Caucasus, Persia and Siberia. It has been introduced to a few places in North America and Australia. Although it often occurs in fields and other habitats influenced by man it is found more often than *V. cracca* in natural plant communities, like in open deciduous forests and in steppe grasslands.

The chromosome number determinations of *Vicia cracca* reported in the literature display an interesting set of data. The first chromosome count of this species was made by SAKAMURA (1914) who found 12 chromosomes in root-tips of a Japanese strain. SVESHNIKOVA (1927) reported counts of ten strains from different parts of Russia and Germany. Only one of them, originating from the province of Moscow, had the diploid number 12, whereas nine of them, one again from the province of Moscow, had 28 chromosomes. SVESHNIKOVA quite logically proposed that a 14 chromosome race would probably exist. Rather soon SVESHNIKOVA (1928) found what she had expected: amongst a large number of seed samples from Europe and Asia one with the diploid chromosome number 14 the rest having 28 chromosomes. The tetraploid strain is thus much more common in the material of SVESHNIKOVA than either of the diploids. On the contrary, RYKA (1954) reported counts of seven samples from southern Poland, only one of which was 28 the remaining six having 14 somatic chromosomes. RENKONEN and THERMAN (1952) reported root-tip counts of twelve seed samples originating mainly from botanical gardens. Eight of them had 28 chromosomes, one had 27, one 24, one 14, and one 12 chromosomes. Dr. THERMAN has very kindly given me a large number of seed samples of *Vicia*, including several of the ones mentioned in the paper of RENKONEN and THERMAN. When plants are grown from the seeds the sample with 24 chromosomes appears to belong to *Vicia tenuifolia* ROTH. Therefore only the numbers 28, 27, 14 and 12 represent *Vicia cracca s. str.* in the material of RENKONEN and THERMAN. BAKSAY (1954) found from Hungary 14 chromosome strains and two populations with 24 chromosomes, which she also included into *V. cracca*. MORIYA and KONDO (1950) report n=7 in a *Vicia cracca* sample which in all probability comes from Japan although its origin is not given quite clearly. The same number is reported by SENN (1938) in a plant at the Blandy Experimental Farm in Boyce, Virginia, U.S.A. The origin of this plant is apparently unknown. Somatic counts of 28 chromosomes in *Vicia cracca* have been reported in material originating from Lund, Sweden (LÖVE and LÖVE, 1944), from Iceland (LÖVE and LÖVE, 1956) and from

Carnduff, Saskatchewan (LEDINGHAM, 1957). The same number was found by JØRGENSEN, SØRENSEN and WESTERGAARD (1958) from Tasiusak in southern Greenland and from Sorgenfri, Zealand in Denmark.

In *Vicia tenuifolia* ROTH only the somatic number of 2n=24 has been reported (HEITZ, 1931; RYKA, 1954; BAKSAY, 1954). It seems to fit into the chromosome number series 12, 14, 28 of *Vicia cracca* as a missing link. It seemed worth while to investigate a number of *V. tenuifolia* samples from different parts of the world to see whether any diploid forms exist. Furthermore it seemed challenging to compare the 12 chromosome strains of *Vicia cracca* with *V. tenuifolia* cytologically and morphologically to see whether one could consider the latter as a polyploid product of the former. A cytological and morphological study of the various chromosome races of *Vicia cracca* as well as a more complete picture of their distribution would perhaps elucidate their origin and their evolutionary status at present. The results of these investigations will be published as a series of papers, beginning with the present one which deals only with the somatic chromosomes.

II. MATERIAL AND METHODS

The material consists of seed samples of *Vicia cracca* and *V. tenuifolia* collected from nature or from various botanical gardens (Table 1). A considerable proportion of the seed samples which came from botanical gardens were erroneously identified, but fortunately the seed of the two species can be identified rather easily. Therefore it was not necessary to grow plants from every seed sample although this was done in most cases.

The seeds were germinated on filter paper or moist sand. The germination took place in a few days if the seeds were scratched with sandpaper, but was otherwise very slow. The seeds of *Vicia tenuifolia* did not germinate as easily as the ones of *V. cracca* on filter paper, but on moist sand, partly submerged, they germinated readily. Perhaps this is caused by the larger size of the seeds of *V. tenuifolia*, the moisture being too much one-sided for them on filter paper.

When a seed germinated the radicle was cut off and made into a squash preparation. Although *Vicia*, like other dicots, produces one single main root when germinating, the root-tip can be cut off without causing damage to the seedling. Secondary roots are formed and the plants can be potted and grown to maturity. This is a great advantage when an especially interesting, for instance aneuploid, chromosome number is found in a particular seedling.

The root-tips were pretreated with 0.05 % colchicine solution for 3 hours or with saturated aqueous paradichlorbenzene solution for 4 hours. After warming for a few seconds in a 9:1 mixture of acetic orcein and 1-n HCl the roots were transferred into tap water. Keeping them overnight in water clearly improved the staining. They could be kept in water for two or three days without any harm. The actual squashing was done in pure acetic orcein. The Feulgen technique after 3:1 fixation was also used and gave very good results in squash preparations.

The colchicine pretreatment spread out the chromosomes very nicely and made the centromere perfectly clear. The secondary constrictions, however, usually disappeared because of the strong contraction of the chromosomes. Therefore other pretreatments were also used. 8-hydroxyquinoline caused numerous crossed stripes to appear in the chromosomes, the centromeres were difficult to discern in some cases and the chromosomes were too long and winding to be analyzed morphologically. Paradichlorbenzene was better than colchicine in making the secondary constrictions visible but did not cause as good spreading of the chromosomes as colchicine.

TABLE 1. *The plant material investigated in the present study.*

The chromosome number determinations made by Dr. EEVA THERMAN are indicated as E.T.

1. *Vicia cracca* L. *Material from nature*

	2n
Finland, Ii, Alaranta, on a river terrace (Herb. Univ. Turku, coll. M. Malvari)	28
„ , Juankoski	28
„ , Jyväskylä	28
„ , Järvenpää	28
„ , Helsinki, Töölö	28
„ , Lahti, Kalliola	28
„ , Velkua, Teersalo, on a gravelly seashore (Herb. Univ. Turku, coll. E. Pertola & T. Laine)	28
Sweden, Skåne	28
Denmark, Langeland, Restinge Klint, seashore meadow	28
„ , Langeland, Gulstav, copse at the seashore	28
„ , Fyn, Tiselholt pr. Vejstrup, hedge	28
„ , Jutland, Vejrs, sand dunes	28, 30
Island, Hueragerdi	28 E.T.
England, Cambridge, Wicken fen	28
„ , Cambridge, Chippenham fen	28
„ , Edinburgh	28 E.T.
„ , Kent	28

	2 n
Holland, N of Hague on dunes	28
Germany	28
„ , Schlüsselburg, Rittergut Schlüsselburg-Neuhof	27, 28
„ , Wilhelmshaven	28
Poland, Krakov distr., Babia-Gora mountains	28
France, environments of Aubusson	14
„ , Coutances, Cametours	28
Switzerland, Altendorf, at the upper part of Lake Zürich	28
„ , 10 km south of Basel between Duggingen and Aesch, on a railroad bank	28
„ , Neuchatel	28
Austria, Graz	14 E.T.
„ , Solbad Hall	28
Hungary, Mátrahaza	14
USSR, Igarka, sandy alluvium near Jenisei	14, 21
„ , in an island of the Lena river 100 km above Jakutsk	14
„ , Serpuhovsk	28
Canada, Ontario, Ottawa, in the city	28
„ , Quebec, Rougemont, on sand	28
„ , Quebec, Rougemont, at the highway	28
„ , Quebec, Rougemont, near Lake Maryville	28
„ , Quebec, S of Montreal, Clarenceville, near Missisquoi Baie	28
„ , Quebec, Montreal, in the downtown	28
„ , New Brunswick, vicinity of Fredericton	28
„ , New Brunswick, West, at River St. John	28
„ , Nova Scotia, northeast of Halifax, Jeddore Harbour	28
„ , Nova Scotia, N of St. Joseph, Antigonish Co.	28
New Zealand	28

2. *Vicia cracca* L. *Material from botanical gardens*

Denmark, Copenhagen	28 E.T.
„ , Aarhus	28
England, Kew Gardens	12 E.T.
Belgium, Gent	28
Germany, Berlin	27, 28 E.T.
„ , Berlin-Dahlem	28
„ , Eberswalde	28
„ , Frankfurt am Main	28
„ , Giessen	28
„ , Köln	28 E.T.
„ , Lippstadt	28 E.T.
„ , Mainz	14 E.T.
Poland, Lodz	14, 27, 28
„ , Poznan	27, 28
„ , Bydgoszcz	14 E.T.
Czechoslovakia, Brno	28
„ , Tabor	28

	2 n
France, Caen	28
„ , Dijon	28 E.T.
„ , Montpellier	28
„ , Rennes	28
„ , Strasbourg	28
„ , Toulouse	28 E.T.
Austria, Wien	28 E.T.
Hungary, Keszthely	28
Yugoslavia, Zagreb	12, 13
USSR, Kiev	14
„ , Moscow	28
Canada, Manitoba	28 E.T.

3. *Vicia tenuifolia* ROTH. *Material from nature*

Sweden, Skåne, St. Hammar, ca. 10 m. alt.	24
France, near Strasbourg, Krafft, in an alluvial meadow on the shore of Rhine	24
Czechoslovakia, Bohemia, Millesov—Lovisberg, copses near the summit	24
Hungary, Budapest, in an open forest	24
USSR, Tadshikistan	24
„ , near Tashkent	24

4. *Vicia tenuifolia* ROTH. *Material from botanical gardens*

Sweden, Stockholm	23, 24
Denmark, Copenhagen	24
Belgium, Brüssel	24 E.T.
France, Rennes	24
Poland, Lodz	24
„ , Poznan	24
Czechoslovakia, Brno	24, 25
Hungary, Budapest	24

III. CHROMOSOME NUMBER AND MORPHOLOGY

1. Euploid numbers

As seen in Table 1, by far the most common somatic chromosome number in the present material of *Vicia cracca* L. is 28. It is the only euploid number hitherto found in the whole of Fennoscandia, England, Holland, Germany, Switzerland, North America and New Zealand. In addition to 28 chromosome strains 14 chromosome ones have been found from France, Austria, Poland (RYKA, 1954) and USSR. No 28 chromosome strains have been found so far from Asia. Remembering the count 2n=14 published by MORIYA and KONDO (1950) from apparently Japanese material and taking into consideration the paucity

of material which I have been able to get from Asiatic sources it seems that the diploid number is more widespread there than the tetraploid one. At any rate, in Asia diploids have been found as far north as the tundras of Siberia, whereas in Europe the northernmost localities so far are in Southern Poland.

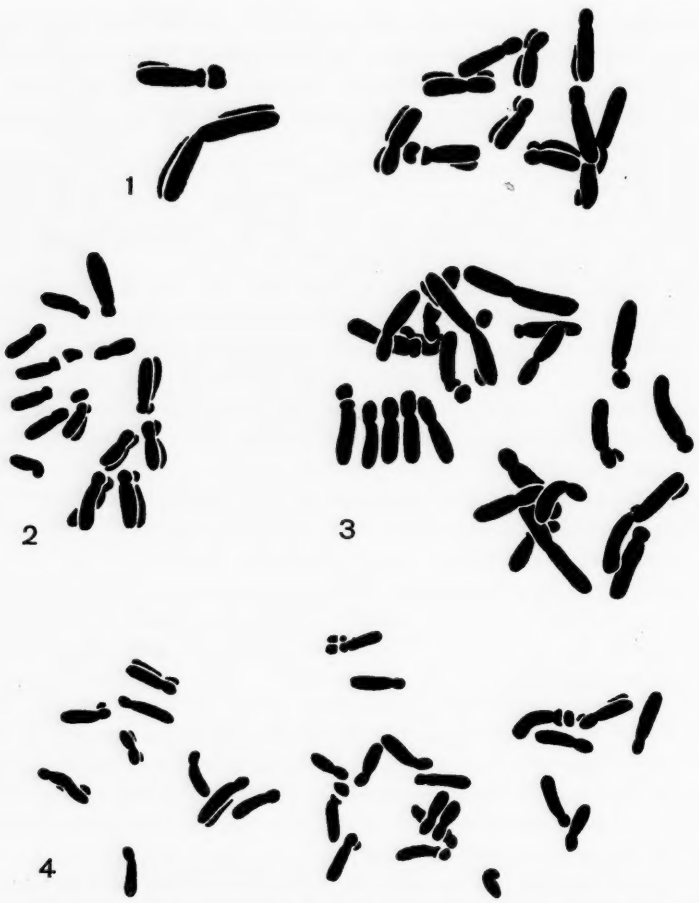
The population grown from the sample of the Lodz botanical garden consisted of both diploid (2n=14) and tetraploid (2n=28) individuals: of 18 plants 10 were diploids and 8 tetraploids. Apparently both types have grown mixed together in the same plot.

The triploid chromosome number (2n=21, Fig. 7) found in the otherwise diploid (2n=14) Igarka sample is represented by a single seedling. Presumably it is a result of an unreduced gamete having taken part in the fertilization. It did not differ to any noticeable extent from the diploid plants during the first summer. In the winter it, however, died, as did part of the diploid individuals. Therefore the reproductive organs could not be studied.

The distribution of the 12 chromosome race is very problematic. The only two records of it from nature are those of SAKAMURA (1914) from Japan and of SVESHNIKOVA (1927) from the province of Moscow. In the material of the present author it is represented by two samples, one from the botanical garden in Zagreb, Yugoslavia and one from the Kew Gardens. In the latter place it has been grown for over 50 years. The curator of the garden, Dr. W. M. CAMPBELL (personal communication) feels sure it was of British rather than foreign origin. It seems, however, to differ markedly from the wild *Vicia cracca* growing in the London area. Although it may originally come from some place in England, it certainly does not represent a common English type of *Vicia cracca*. Other evidence suggests that the 12 chromosome race probably has a much more eastern distribution.

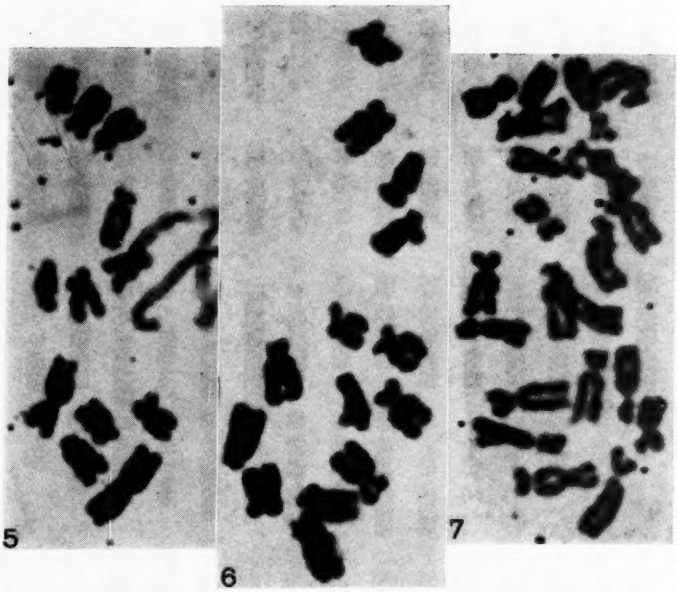
As contrasted by the variable chromosome number of *Vicia cracca*, *V. tenuifolia* displays one euploid number only, viz. 2n=24 in all of the 14 strains investigated cytologically. This agrees with the results obtained by HEITZ (1931) and RYKA (1954). The morphology of somatic chromosomes was studied thoroughly in two strains with 12 chromosomes, in four strains with 14 chromosomes, in five strains with 28 chromosomes and in two *Vicia tenuifolia* strains with 24 chromosomes. Idiograms of all these strains were made and compared. In addition, more superficial observations on the karyotype were made together with the chromosome number determinations.

The chromosome complements of the *Vicia* strains investigated have



Figs. 1—4. Camera lucida drawings of root-tip metaphase cells pretreated with paradichlorbenzene and stained with Feulgen. — Fig. 1. *Vicia cracca* from Kew, 2n=12. — Fig. 2. *V. cracca* from Lodz, 2n=14. — Fig. 3. *V. tenuifolia* from Brno, 2n=24. — Fig. 4. *V. cracca* from Velkua, 2n=28. — × 2200.

few clear landmarks which would help in distinguishing the chromosome pairs from each other. Secondary constrictions could be seen in only one pair of chromosomes in the diploids and in two pairs of chromosomes in the tetraploids (including *V. tenuifolia*). This chromosome pair always looked very similar when it could be discerned, indi-



Figs. 5—7. Photomicrographs of root-tip metaphase cells pretreated with colchicine and stained with acetic orcein. The black dots are orcein crystals. — Fig. 5. *Vicia cracca* from Zagreb, 2n=12. The two prophase chromosomes belong to a neighbouring cell. — Fig. 6. *V. cracca* from Aubusson, 2n=14. — Fig. 7. *V. cracca* from Igarka, a triploid individual with 2n=21. — × 2600.

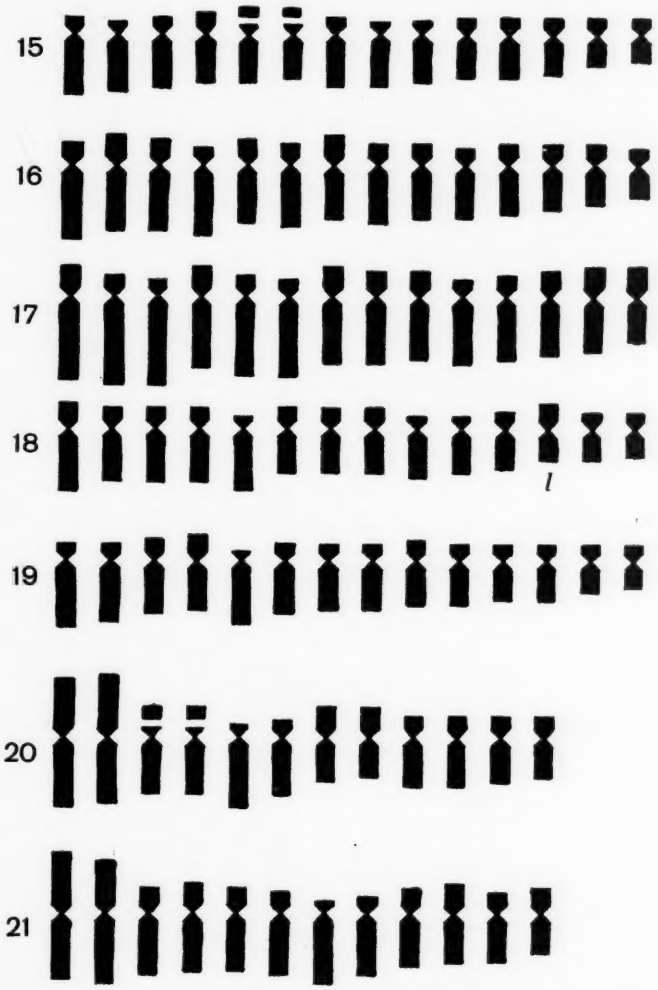
cating that it has not taken part in any major chromosomal rearrangements when the karyological differences between the strains have arisen. The secondary constriction has a strong tendency to disappear when the chromosomes are fully contracted in mid-metaphase. This happens regularly when chromosomes are pretreated with colchicine. The constriction can be seen more often if paradichlorbenzene is used as pretreatment but even then it very often disappears into the cromatin mass. In some of the karyotype analyses the secondary constrictions were not taken into consideration because of the difficulty in discerning them and because they do not seem to be of any importance in distinguishing the karyotypes.

In the 12 chromosome strain of *Vicia cracca* one chromosome pair can be at once distinguished from the other chromosomes. It is the longest pair in the set, characterized by two about equally long arms (Figs. 1, 5, 12, and 13). Already SVESHNIKOVA (1928) pointed out the

Figs. 8—14. Idiograms measured from root-tip metaphase cells. The chromosomes are arranged in a decreasing size order. — Fig. 8. *Vicia cracca* from Lodz, 2n=14. — Fig. 9. *V. cracca* from Bydgoszcz, 2n=14. — Fig. 10. *V. cracca* from Jakutsk, 2n=14. — Fig. 11. *V. cracca* from Igarka, 2n=14. — Fig. 12. *V. cracca* from Kew, 2n=12. — Fig. 13. *V. cracca* from Zagreb, 2n=12. — Fig. 14. The idiogram of the unknown male parent of the 13 chromosome hybrid seedlings from Zagreb. — × 2600.

existence of such a chromosome in this race. If one compares the karyotypes of the 12 and 14 chromosome races certain differences can be seen easily. The long metacentric chromosome pair of the 12 chromosome race is substituted by two subacrocentric pairs with the longer arms roughly equal to the arms of the metacentric chromosome. These two chromosome pairs are the longest ones in the 14 chromosome strains (Figs. 8—11). Another visible difference is in the smallest chromosome pair which is somewhat more metacentric in the 14 chromosome race than in the 12 chromosome one. In the four strains with 14 chromosomes the proportions of arms were measured from camera lucida drawings as 6:4 (Lodz), 8:6 (Igarka), 8:5 (Jakutsk) and 8:6 (Bydgoszcz). In the two 12 chromosome strains the corresponding figures were 9:4 (Kew) and 10:3 (Zagreb).

The small differences in the karyotypes of the two 12 chromosome strains as well as those in the karyotypes of the four 14 chromosome strains are probably not significant. Small fluctuations in the length of

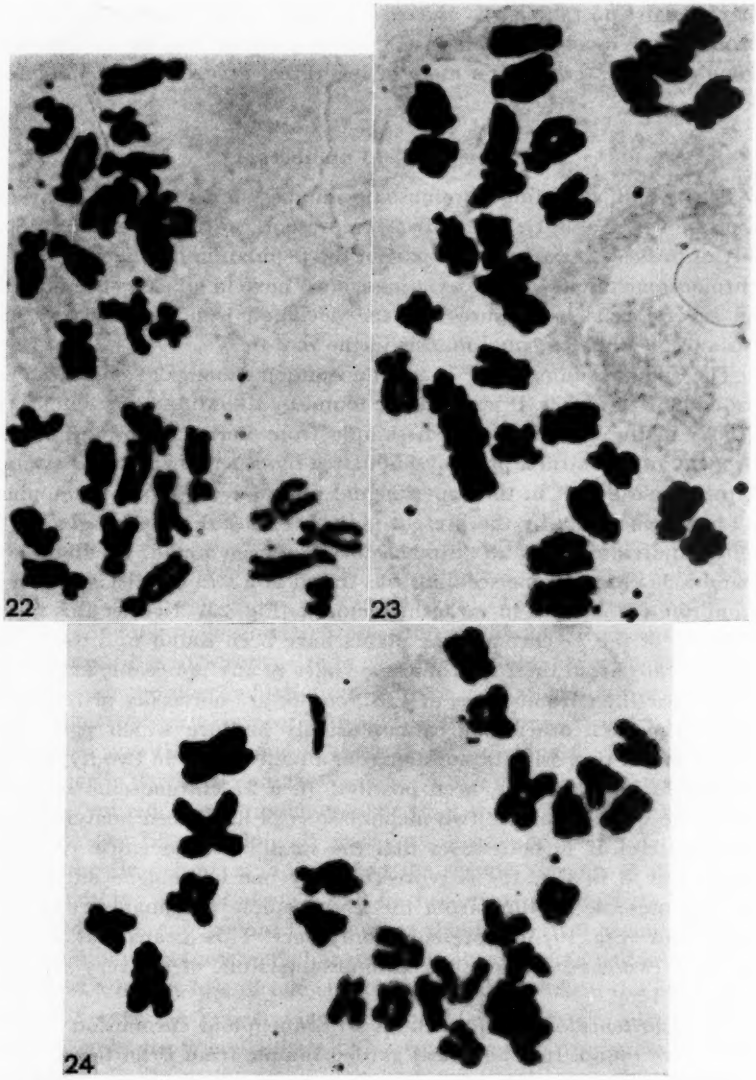


Figs. 15—21. Idiograms measured from root-tip metaphase cells (continued). — Fig. 15. *V. cracca* from Velkua, 2n=28. — Fig. 16. *V. cracca* from Berlin, 2n=28. — Fig. 17. *V. cracca* from Schlüsselburg, 2n=28. — Fig. 18. *V. cracca* from Poznan, 2n=27. — Fig. 19. *V. cracca* from Lodz, 2n=27. — Fig. 20. *V. tenuifolia* from Brno, 2n=24. — Fig. 21. *V. tenuifolia* from Stockholm, 2n=23. — × 2600.

the chromosomes always occur, as is easily shown if two metaphase cells of the same individual are analyzed. Such small differences can be caused by an unequal squashing or a different degree of contraction depending upon the intensity of the pretreatment, certain chromosome arms being contracted proportionally more than others. In fact, most of the chromosomes are impossible to identify with certainty. They are nearly of the same shape, only forming a gradient in respect of their size. Sometimes, however, a chromosome pair can be recognized by its centromere being more terminal or more median than in the other chromosome pairs in the same size class.

If one compares the chromosome complements of the 14 and 28 chromosome races (Figs. 8—11 and 15—19) they are essentially very similar. For example, the satellited chromosome pair of the 14 chromosome race is represented by two similar pairs in the 28 chromosome race. However, all chromosomes of the tetraploid race cannot be combined into groups of four equal chromosomes, which would be the case in a completely autotetraploid race. For instance the chromosome 1 in the Poznan strain (Fig. 18) is clearly different from others in the set in being almost perfectly metacentric. Other differences in chromosome pairs can be found in the idiograms (Figs. 15—19), although one has to remember that, as mentioned earlier, small differences in length of a chromosome arm can be caused by, *e.g.*, the pretreatment or the amount of squashing. In any case it can be concluded that although the 28 chromosome race has not changed its karyotype much in evolving from the 14 chromosome race, minor rearrangements have taken place at least in some cases. Small karyotype differences between various 28 chromosome strains seem to exist although they are difficult to point out or classify exactly. The differences in absolute length of the chromosomes, which can be observed when the various karyotypes are compared (Figs. 15—19) are, however, probably caused by a different degree of contraction, which again depends on the intensity of the pretreatment and the exact stage of metaphase in the analyzed cell.

The karyotype of *Vicia tenuifolia* (Figs. 20, 21) is very similar to that of the 12 chromosome *V. cracca* race. The chromosomes of *V. tenuifolia* can be divided into groups of four, each group being very similar to a pair of chromosomes of the 12 chromosome *V. cracca*. The small discrepancies which exist can be caused by similar accidental factors as mentioned earlier and do not have anything to do with chromosome rearrangements. This means that on the basis of chromosome morphology *V. tenuifolia* could even be a direct autotetraploid product of the



Figs. 22—24. Photomicrographs of root-tip cells of aneuploid individuals. The cells are pretreated with colchicine and stained with acetic orcein. The black dots are orceine crystals. — Fig. 22. *Vicia cracca* from Lodz, 2n=27. — Fig. 23. *V. cracca* from Jutland, 2n=30. — Fig. 24. *V. tenuifolia* from Brno, 2n=25. — × 2600.

12 chromosome race of *V. cracca*. Although simple autopolyploidy is probably not involved in this case, it seems possible that the 12 chromosome race of *V. cracca* is a more or less direct predecessor of *V. tenuifolia*.

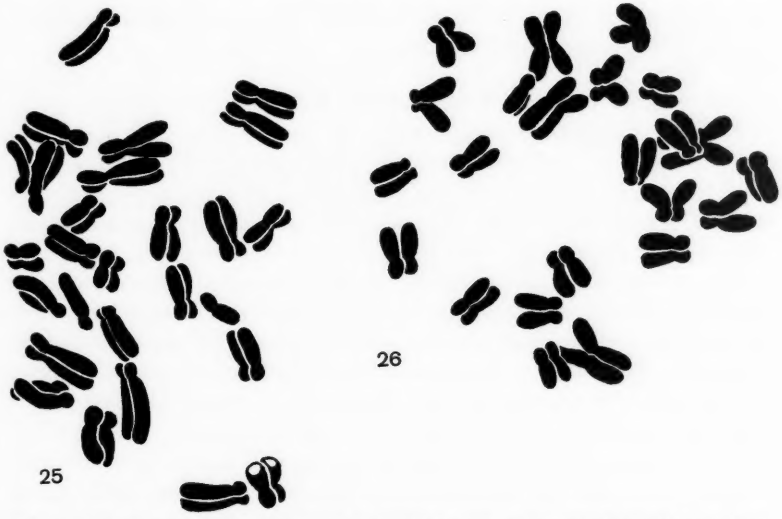
2. Aneuploid numbers

Plants with deviating chromosome numbers are found in many of the samples (Table 1). They always represent, however, a small minority of the seedlings the major part of the population having an euploid chromosome number. The deviating counts have in all cases been based on several cells to be sure that the deviation is not caused by, for instance, a single unequal mitosis in the root-tip.

The somatic number 27 is not uncommon among 28 chromosome individuals (Fig. 22). It was already found by RENKONEN and THERMAN (1952) in the botanical garden sample from Berlin. However, in the progeny of this strain, possibly pollinated by other *Vicia cracca* strains growing close to it in the experimental plot, only the somatic number 28 has been found by the present author. — Peculiarly enough, not a single individual with 29 chromosomes has been found. On the other hand a 30 chromosome seedling was found in a strain collected from a sand dune in Jutland in western Denmark (Fig. 23). Neither this plant nor any of the 27 chromosome plants have been found to differ morphologically from the 28 chromosome ones to any noticeable extent.

Because the chromosomes of *Vicia cracca* are not easily distinguishable from each other, one cannot usually be sure which particular chromosome is missing or additional in an aneuploid. In two hypoploid cases this has, however, been possible. In a 27 chromosome seedling from the Poznan sample two metaphase cells have been analyzed and it is quite clear in both cases that the small submetacentric chromosome pair (1 in Fig. 18) is represented by one homologue only. In a 27 chromosome seedling from the Lodz sample the smallest chromosome pair (Fig. 19) is represented only once. This means that at least in these two cases the missing chromosome is one of the very smallest ones.

In *Vicia tenuifolia* two seedlings with aneuploid chromosome numbers were found. In a botanical garden sample from Brno there was a plant with 2n=25 (Fig. 24), in a sample from the Stockholm botanical garden a plant with 2n=23 (Fig. 26). Which chromosome is additional in the former one or which is missing in the latter one could not be decided. The only thing one can be certain about is that it is not any



Figs. 25—26. Camera lucida drawings of root-tip cells of aneuploid individuals. Pretreatment with colchicine. — Fig. 25. *Vicia cracca* from Poznan, 2n=27. — Fig. 26. *V. tenuifolia* from Stockholm, 2n=23. — × 2000.

of the four long metacentric chromosomes. The 23 chromosome individual looked even stronger than the two individuals with a normal chromosome number which I grew from the Stockholm sample. It was larger in every respect during the first summer and, strangely enough, it was the only one of these three individuals which survived the winter in Turku Botanical Garden. On the other hand the 25 chromosome individual was much weaker than the four with 24 chromosomes in the Brno sample. It was very weak and had small leaves during the first summer, and during the second summer it died. Unfortunately the control plants I had in these two samples were too few for any definite conclusions to be drawn about the effect of aneuploidy. It seemed obvious, however, that the extra chromosome had a deleterious effect whereas loss of one chromosome did not harm the plant the least. Whether the greater vigour of the 23 chromosome plant really was caused by the aneuploidy is, of course, uncertain. It may be a result of a mere chance in the small sample. Interestingly enough, however, a somewhat similar effect of aneuploidy is shown by VAARAMA (1953) in the progeny of an autotetraploid *Ribes nigrum*. The 4x—1 types were in some respects, *e.g.* in rate of growth, more vigorous than the

4x types, whereas the 4x+1 individuals had a somewhat decreased vigour as compared with the 4x types.

3. A spontaneous hybrid

In the *Vicia cracca* sample from the Zagreb botanical garden in which 12 was the normal somatic chromosome number, seven seedlings with 13 chromosomes were found (Fig. 27). One might expect these to have six normal chromosome pairs and one extra chromosome. The chromosome morphology was, however, quite different: the long metacentric chromosome pair of the 12 chromosome plants was represented in the 13 chromosome seedlings by one chromosome only. This could be seen very easily in all seven individuals with 13 chromosomes. It seems likely that they are hybrids between the 12 chromosome mother plants and some 14 chromosome father plants which did not have a long metacentric chromosome pair.

The chromosome complements of the 13 and 12 chromosome individuals were carefully analyzed. From the 13 chromosome set those six chromosomes were deducted which were found to be equal to the haploid complement of the 12 chromosome individuals. Fig. 14 shows the remaining seven chromosomes, in other words the haploid complement of the unknown male parent. It is very similar to the karyotype of the 14 chromosome race of *Vicia cracca* (Figs. 8—11). Because a 14 chromosome race could very well occur in Zagreb, perhaps as a weed in the botanial garden, the pollination of a 12 chromosome plant with a 14 chromosome *Vicia cracca* would be quite possible. The karyotypes of the various *Vicia* species are, of course, not well enough known to exclude other species as possible pollinators. On the other hand, clear-cut karyological differences do exist among the various species of *Vicia* (SVESHNIKOVA, 1927; HEITZ, 1931; CÂMARA and COUTINHO, 1935) and no species with exactly the same karyotype as *V. cracca* is known.

Rather strong evidence in favour of the 14 chromosome race of *Vicia cracca* being the male parent comes from a study of the serological properties of the seeds. As was first shown by RENKONEN (1948) the seeds of some leguminous plants contain substances which agglutinate human red cells specifically according to the blood group. RENKONEN and THERMAN (1952) showed that the agglutinins in the seeds of the 12 chromosome *Vicia cracca* strain studied (as well as of *V. tenuifolia*) were unspecific, whereas the 14 and 28 chromosome strains contained a strong anti-A_1-agglutinin. The present author determined chromo-

Fig. 27. The somatic chromosomes of a hybrid seedling with 2n=13 from Zagreb. Note one long metacentric chromosome. Pretreatment with paradichlorbenzene, staining with Feulgen. — × 2200.

some numbers of a small sample of Zagreb seeds, separated the ones with 12 and 13 chromosomes and tested the agglutinins present in the two seed groups. An extract was made of both groups of seeds in physiological NaCl-solution, the weight ratio of seeds and NaCl-solution being 1:20. After centrifugation twofold serial dilutions were made from both extracts. The extract from the 13 chromosome seeds showed a clear anti-A_1-specifity in three strongest concentrations, whereas the corresponding extract from the 12 chromosome seeds did not cause any agglutination in even the strongest concentration. This result strongly suggests that the 13 chromosome seeds are a result of hybridization, the male parent having contained a specific anti-A_1-agglutinin. According to MÄKELÄ (1957), of 61 *Vicia* species tested serologically, in addition to *V. cracca* only four (two of them questionably) show anti-A_1-specifity. This evidence, combined with the evidence presented by the karyotype, minimizes the possibility of some other *Vicia* species being the male parent. — Because the F_1 seeds show anti-A_1-activity this property is probably caused by one dominant gene. A genic control of the agglutinin formation was shown for the first time in a recent publication by SCHERTZ, JURGELSKY and BOYD (1960) who presented strong evidence for high anti-A_1 hemagglutinating activity in Lima beans being caused by a dominant gene.

In all probability, therefore, the 13 chromosome seedlings represent spontaneous hybrids between the 12 and 14 chromosome races of *Vicia cracca*. From this point of view it was particularly interesting to note that all seedlings with 13 chromosomes were albinos with no chlorophyll formation (Fig. 28). They died after a few weeks and did not grow more than a few centimeters tall. Interestingly enough, SVESHNIKOVA (1929) got one 13 chromosome seedling from the seeds of the 12 chromosome *Vicia cracca* strain which she had grown in her experimental plot. From the morphology of the chromosomes and from the

fact that the 14 chromosome race and the 12 chromosome race grew rather close to each other in her experimental field she concluded that the 13 chromosome plant was a hybrid between the two diploid chromosome races of *Vicia cracca*. Furthermore she mentions that the hybrid seedling became etiolated and destroyed, which very probably means the same albino condition as in the 13 chromosome seedlings found by the present author.

IV. DISCUSSION

1. Geographical distribution of the chromosome races

It is quite obvious from Fig. 29 that the various chromosome races of *Vicia cracca* have a clearly different geographical distribution. The 14 chromosome race is distinctly more southern and eastern than the 28 chromosome race. Although the former extends even north of the arctic circle in Siberia it is missing altogether from the more northern parts of Europe.

It is now a well known fact that in the northern hemisphere a greater percentage of polyploids is found in the floras of more northern regions than in those of the more southern ones (*e.g.* LÖVE and LÖVE, 1953, 1957; REESE, 1957). On the other hand, in a species or a group of

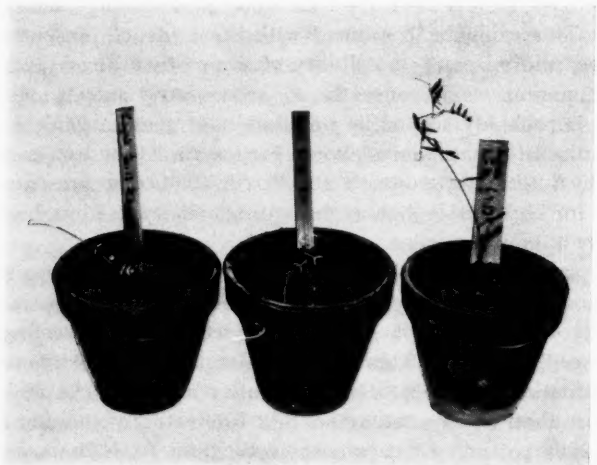


Fig. 28. From the left, two individuals with 2n=13 and one with 2n=12, all from the same seed sample (Zagreb) and grown in the same conditions.

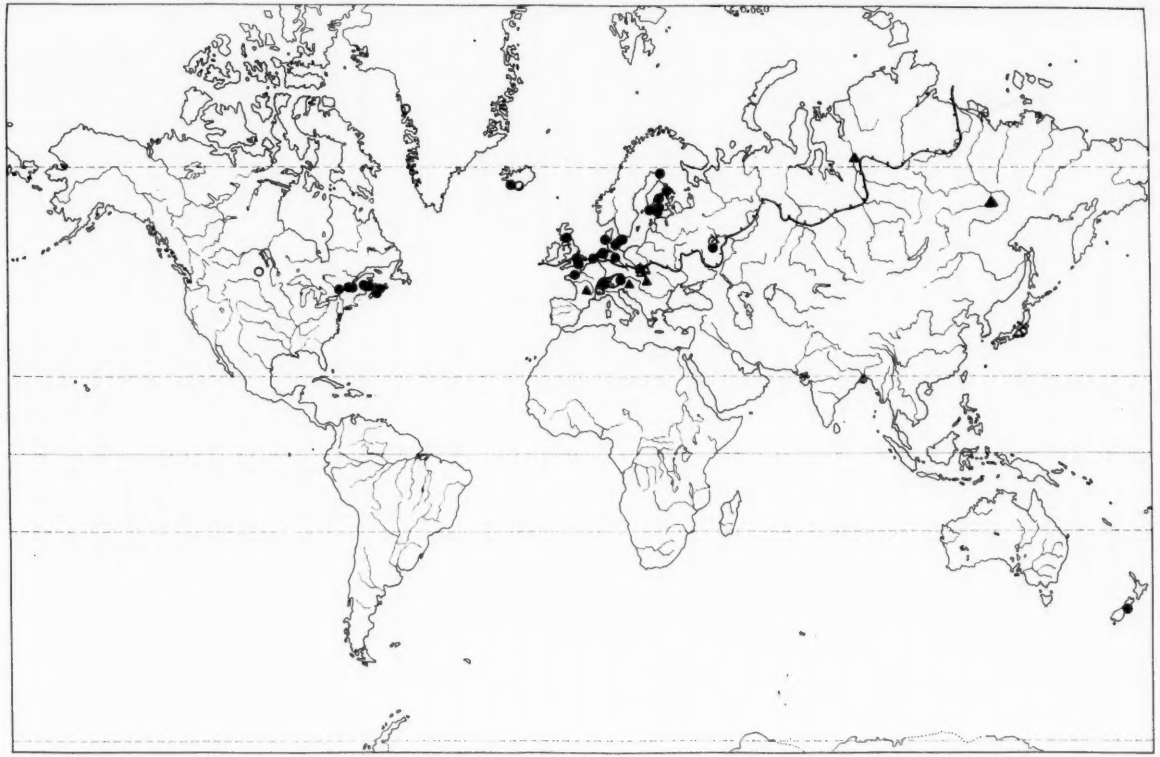


Fig. 29. The known localities of the various chromosome races of *Vicia cracca*. Only the samples collected from nature are included. Black circles: 2n=28 according to the present author. White circles: 2n=28 according to the literature. Black triangels: 2n=14 according to the present author. White triangels: 2n=14 according to the literature. Crosses: 2n=12 according to the literature. The maximum extent of Pleistocene glaciation in Europe and Siberia is shown by a hatched line.

closely related species where both diploids and polyploids occur the polyploids do not generally have a more northern distribution than the diploids (STEBBINS, 1950). The high incidence of polyploids in the more northern floras is believed by some authors (*e.g.* LÖVE and LÖVE, 1953, 1957) to be caused, at least partly, by the polyploids having a greater tolerance for extreme climatic conditions, a hypothesis originally put forward by HAGERUP (1932). STEBBINS (1942, 1950), on the other hand, followed by perhaps most authors nowadays, relates this phenomenon to the better colonizing ability of the more recent polyploid forms as compared with their diploid predecessors and relatives. The occupation of the available habitats after the retreat of the pleistocene ice sheet would have been done more effectively by polyploids than by diploids, this phenomenon causing the increase of the percentage of polyploids when one goes northward in Europe. In fact, the better colonizing ability of the polyploids as compared with their diploid ancestors has been supported by a number of investigations (ANDERSON, 1936; ANDERSON and SAX, 1936; BABCOCK and STEBBINS, 1938; CLAUSEN, KECK and HIESEY, 1940, 1945; MANTON, 1934, 1937). It seems very probable that this historical factor is the main reason for the greater percentage of polyploids in the northern parts of Europe, although there is some evidence, too, for a greater hardiness of the polyploids as a partial reason for this phenomenon. This can be deduced from the exceptionally high frequency of polyploids in the subarctic and arctic islands which have been separated from the mainland since the last glaciation and where plants are believed to have survived during that time in unglaciated refugia (LÖVE and LÖVE, 1953, 1957).

In the case of *Vicia cracca* the distribution of the 14 and 28 chromosome races in Europe seems to be largely determined by the maximum extent of the pleistocene glaciation. Although one cannot draw very definite conclusions because of the paucity of the material available, it seems rather safe to believe that the 14 chromosome race is totally restricted to areas not covered by the ice sheet. Interestingly enough the district of Krakow in Poland where the distribution areas of the 14 and 28 chromosome races overlap is just at the edge of the maximum ice cover. It is probably significant that Switzerland and western Austria are represented by tetraploid populations only whereas other populations in the same latitude are diploid. This area now occupied by tetraploid populations was covered during maximum glaciation by an ice sheet originating from the Alps. The situation resembles the famous examples of *Biscutella laevigata* (MANTON, 1934, 1937) and *Iris versi-*

color (ANDERSON, 1936). The much greater aggressiveness of the 28 chromosome race as compared with the 14 chromosome one in occupying newly available habitats is no doubt the most natural explanation for their distribution picture in Europe. It may very well be that *Vicia cracca* like many other plants now mainly occurring in habitats produced by man's action was common during the Late-glacial period when the newly available areas were not yet covered by forests, was largely suppressed by the invading forests but became common again when man's action became more prominent and produced more open habitats. Weeds and ruderals which show this kind of distribution history are very numerous. In Britain *e.g. Cirsium heterophyllum* (L.) HILL, *Leontodon autumnalis* L., *Plantago major* L., *Ranunculus acris* L., *Taraxacum officinale* L. *coll.* and many other trivial plants belong to this group (GODWIN, 1956). The ability of the 28 chromosome *Vicia cracca* to occupy habitats newly released from the ice cover would then largely determine its recent distribution. The 14 chromosome race has apparently not, despite of its weedy nature, expanded much northwards since the glaciation.

It is interesting to note that the 28 chromosome race is as far as is known the only one of the three chromosome races of *Vicia cracca* which has spread to other continents, namely North America and New Zealand. This fact fits very well into the picture of it as the pioneer race of the species.

In Asia the 14 chromosome race extends much more northwards than in Europe, *e.g.* the Igarka sample was collected north of the arctic circle. It is interesting to note, however, that the maximum pleistocene glaciation extended very little outside the Igarka locality and did not cover at all the Jakutsk locality where the 14 chromosome race was also found. Even the Igarka locality was outside the ice sheet during the latest glacial maximum (FLINT, 1957). Unfortunately the available Asiatic material of *Vicia cracca* is so scanty that it does not much elucidate the distribution of the various chromosome races there. It is not impossible, however, that the extension of the 14 chromosome race so far north in Asia has something to do with the fact that glaciation did not play as important role there as it did in Europe, large parts of Siberia being totally unglaciated (FLINT, 1957). The 28 chromosome race is not found in Asia so far but it would be most interesting to see whether it could be found in the regions once glaciated.

2. Karyotype evolution

Vicia cracca and *V. tenuifolia* represent an interesting series of karyotypes, the former having three chromosome races with 12, 14 and 28 chromosomes, the latter completing the series with a somatic number 24. Whereas within each of these four types little or no variation in the chromosome morphology can be detected, two clearly different karyotype categories are recognizable. The 12 chromosome race of *Vicia cracca* and all *V. tenuifolia* strains studied have essentially the same karyotype, and the same is true with the 14 and 28 chromosome races on the other hand.

The most conspicuous karyological difference between the 14 and 12 chromosome races is the substitution of two long subacrocentric pairs of the former by a long metacentric pair in the latter. In other words the number of long chromosome arms is the same in both although the number of chromosomes is different. As was experimentally shown first by DUBININ (1936, cited by BABCOCK, 1947) and independently suggested by DARLINGTON (1937), a change in basic chromosome number can be caused by a reciprocal translocation and a subsequent loss of a chromosome consisting of heterochromatic regions. This kind of karyotype evolution has been shown to occur in many kinds of organisms, the origin of *Crepis fuliginosa* from *C. neglecta* (TOBGY, 1943) being one of the most beautiful cases in the plant kingdom. A special type of these translocations, called centric fusions, is known to be prevalent in many animals, such as grasshoppers and reptiles (WHITE, 1954). This means converting acrocentric chromosomes into metacentric ones by translocations involving entire euchromatic arms. As a byproduct a genetically more or less inert "dwarf" chromosome arises but is soon lost. In its strict sense, centric fusion seems to be very uncommon in the plant kingdom, the translocations being in most plants less methodical (*cf.* STEBBINS, 1950). Furthermore it has been shown that in plants at the same time as the basic numbers have a tendency to decrease there is a trend for asymmetrical karyotypes (with more acrocentric chromosomes) to evolve from symmetrical ones (with more metacentric chromosomes) which means the reverse of the situation usually found in animals (STEBBINS, 1958). The Gymnosperm family *Podocarpaceae* seems to form an exception in the plant kingdom by displaying a karyotype evolution very similar to the centric fusion of animals. The most primitive types of the family presumably had 20 pairs of rod-shaped chromosomes and the recent ones show a vari-

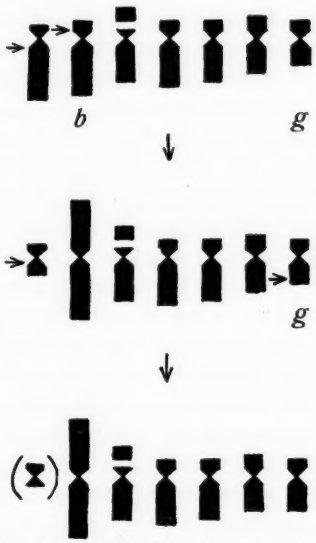


Fig. 30. The hypothetical mode of origin of the 12 chromosome race of *Vicia cracca* from a 14 chromosome race of the same species (the idiogram of the Lodz sample representing the 14 chromosome race). The supposed points of breakage are indicated by arrows. — × 2600.

able number of metacentric chromosomes the number of chromosome arms being, however, constantly 20 (HAIR and BEUZENBERG, 1958).

The karyotype differences of the 14 and 12 chromosome races of *Vicia cracca* suggest that a fairly typical centric fusion has taken place. There is every reason to believe that 7 is the original basic number in the genus *Vicia* (*cf.* SENN, 1938). According to DARLINGTON and WYLIE (1955) 30 species of *Vicia* have 7 as their basic number, 4 species have both 7 and 6, 14 species have 6 alone and 3 species have both 6 and 5. In the other genera of the tribe *Viciae*, *Lens*, *Pisum* and *Lathyrus* have x=7 as their only basic number, whereas *Cicer* has both 7 and 8. It would hardly be possible that 6 would be an older basic number in this group of plants than 7. SVESHNIKOVA (1927) first assumed, before she knew that a 14 chromosome race of *Vicia cracca* existed, that the 12 chromosome race was the primitive one and the 28 chromosome race had been derived from it by segmentation and polyploidy. For various reasons later SVESHNIKOVA (1937) considered the 14 chromosome race as the original one and the 12 chromosome race as derived.

The 12 chromosome race has thus most probably arisen from the

14 chromosome race by a centric fusion type of interchange of the two longest chromosomes (Fig. 30). Another karyological difference that can be easily detected is in the smallest chromosome pair (g in Fig. 30) which is more nearly metacentric in the 14 chromosome race than in the 12 chromosome one. This could be the result of a pericentric inversion. As is suggested in Fig. 30, it could also be a result of another interchange in which a distal part of the recently formed "dwarf" chromosome would have been transferred to the longer arm of the g chromosome. The distal end of the shorter arm of the chromosome b would then have been saved in the chromosome complement and the "dwarf" chromosome, going to waste, would be still smaller.

In the hypothesis presented above, the assumption is made that the 14 chromosome race that gave rise to the 12 chromosome race had the same karyotype as it now has. This is supported by the fact that essentially the same karyotype is found in all the 14 chromosome strains studied although they come from widely different areas. Therefore in the evolution of the 14 chromosome race itself little or no karyotype change seems to have occurred.

The decrease of basic chromosome number has played an important role in the evolution of a number of *Vicia* species. In most cases, however, it is a result of some other kind of rearrangement than a centric fusion type of interchange. SVESHNIKOVA (1927) studied karyotypes of 25 *Vicia* species, 15 with 2n=14 and 10 with 2n=12. She separated the chromosomes into four groups, namely the metacentric ones (ratio of the arm lengths less than 2:1), the submetacentric ones (the above ratio equal or more than 2:1), chromosomes with "heads" and chromosomes with "head" and satellite. The two last named groups together could be called subacrocentric chromosomes. If we calculate on the basis of SVESHNIKOVA's karyotype illustrations the percentage of the various chromosome types in the two groups of species, we find: Of the 105 different chromosomes in the 14 chromosome species, 29 (27.6 %) belong to the metacentric group, whereas in the 12 chromosome species only 8 (13.3 %) of the 60 different chromosomes are metacentric. The number of submetacentric chromosomes in the two species groups are 32 (30.5 %) and 18 (30.0 %) and the number of subacrocentric chromosomes 44 (41.9 %) and 34 (56.7 %), respectively. In other words, instead of having proportionally more metacentric chromosomes the 12 chromosome species have a larger percentage of subacrocentric chromosomes than the 14 chromosome ones. These figures strongly indicate that the prevalent way of decreasing the basic chromosome number in

the genus *Vicia* is not a centric fusion type of interchange. In *Vicia faba*, on the other hand, the karyotype with 5 acrocentric pairs and one long metacentric pair (McLEISH, 1953) suggests that it could be the result of a similar centric fusion as in *Vicia cracca*.

As is evident from the above figures, karyotype evolution in the genus *Vicia* as a whole fits very well into the evolutionary trend shown by many plants, namely an increasing karyotype asymmetry together with a decrease of basic number (STEBBINS, 1950, 1958). On the other hand the statement quoted by some authors (*cf.* STEBBINS, 1950; SWANSON, 1958) that the perennial species of *Vicia* have mostly symmetrical, the annual ones mostly asymmetrical karyotypes is somewhat incorrect, as is shown by SVESHNIKOVA's (1927) data: the section *Ervum* which consists of only annual species has a much greater portion of metacentric chromosomes than species in other sections; in the section *Cracca* where both annuals and perennials occur the five annuals studied by SVESHNIKOVA have, on an average, a more symmetrical karyotype than the four perennials in her material; conversely, the section *Eu-Vicia*, consisting of mostly annual species, has a preponderance of asymmetrical karyotypes, with acrocentric and subacrocentric chromosomes being more common than in the other two sections.

The spontaneous hybrid seeds from the Zagreb sample, probably originating from a hybridization between the 12 and 14 chromosome races of *Vicia cracca*, is a most interesting natural experiment which the present author has failed to accomplish artificially. Because of the albino condition and the resulting inviability of the hybrids it is obvious that the two races have diverged genetically to a considerable extent. A reproductive isolation has formed a barrier between them, probably in the form of genic inbalance between the two genomes. In this connection it is interesting to note that all hybridizations between *Vicia* species have proved extremely difficult. Thus v. SCHELHORN (1940) tried to cross various *Vicia* species with practically no success. Even crossing of two forms of the same species sometimes proved extremely difficult. For example, a cross between two varieties of *Vicia narbonensis* L. with the same chromosome number yielded only a few weak and chlorotic plants which died before reaching maturity. Similarly, SVESHNIKOVA (1927) reports unsuccessful attempts to cross different *Vicia* species. Only the cross between *V. villosa* ROTH. and *V. dasycarpa* TEN. gave seeds. Although these species are so closely related that they very often are combined as one species, the hybrid seedlings were chlorotic and lethal.

Hence it seems that a reproductive isolation in form of hybrid inviability arises very easily between races of *Vicia* species. Hybridization thus seems to be largely prevented as an evolutionary mechanism in the genus. This may be one reason for the quite limited importance of polyploidy in the evolution of *Vicia*.

The tetraploid race of *Vicia cracca* very probably has arisen as a polyploid product of the 14 chromosome race although the kind of polyploidy involved cannot be inferred from the chromosome morphology alone. Further studies, especially analysis of the meiosis will be necessary before any definite conclusions can be made about the mode of origin of the tetraploid race. On the basis of its wide distribution and successful ecological capacities it can be deduced, however, that it probably is not a pure autopolyploid. The autopolyploids are, as is well known, very stable entities in evolution because of the multiple presence of every gene. The karyotypes, too, suggest at least some deviation from pure autopolyploidy. On the other hand, the common occurrence of aneuploid, especially hypoploid forms would probably not be possible in a typical allopolyploid. Accordingly there probably must be considerable gene duplication present in the tetraploid *Vicia cracca*.

Similarly, from the evidence available it is not possible to draw any definite conclusions about the origin of *Vicia tenuifolia*. The chromosome morphology suggests an origin from the 12 chromosome race of *Vicia cracca*. For the same reasons as presented above for the tetraploid *Vicia cracca*, namely the wide distribution and ecological success on the one hand and the occurrence of the aneuploids on the other hand, it does not seem likely that *Vicia tenuifolia* would be either a strict autopolyploid or a strict allopolyploid. As a matter of fact very few plants can be said to belong to either of these groups in their strictest sense.

According to DARLINGTON and WYLIE (1955) the chromosome numbers are known in 51 *Vicia* species which is about one third of all the species in the genus. In addition to *Vicia cracca* and *V. tenuifolia* only three species are known to contain polyploid forms. These are *Vicia amoena* FISCH., *V. unijuga* BR. and *V. alpestris* STEV., which all are Asiatic species with a more limited distribution than the two tetraploids of the present investigation. With the paucity of the polyploids in the genus it is interesting to note that the two tetraploids in question are much more successful types than their diploid predecessors. What the unusual power of these two tetraploids depends on, is a difficult question. It seems that their basic genomes which still are probably

genetically rather similar, are more suitable for polyploidy than those of the other *Vicia* species.

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SUMMARY

44 strains of *Vicia cracca* L. collected from the wild and 29 strains collected from botanical gardens were investigated in regard to the number and morphology of somatic chromosomes. The same was done with 6 strains of *Vicia tenuifolia* ROTH collected from the wild and 8 strains collected from botanical gardens.

In *Vicia cracca* three chromosome races were found, with 12, 14 and 28 somatic chromosomes, respectively, whereas all strains of *V. tenuifolia* had 24 somatic chromosomes. Seedlings with deviating chromosome numbers were found occasionally. Altogether, the following somatic numbers were found in seedlings of *Vicia cracca*: 12, 13, 14, 21, 27, 28, and 30. Similarly in *V. tenuifolia* the numbers 23, 24 and 25 were found. The occasional individuals of *V. cracca* with 27 or 30 chromosomes did not differ morphologically to any noticeable extent from the ones with 28 chromosomes. In *V. tenuifolia* the hypoploid was superior, the hyperploid inferior to the euploids in respect of the vigour.

There is strong evidence from chromosome morphology and agglutinin content that the 13 chromosome seedlings found in a 12 chromosome sample of *V. cracca* were spontaneous hybrids between this 12 chromosome strain and some 14 chromosome plant of *Vicia cracca*. These hybrid seedlings were albinos and died in a few weeks after germination.

The geographic distribution of the chromosome races is discussed. In Europe the 14 chromosome race is more southern than the 28 chromosome one and has not been found, so far, in the area covered by the maximum Pleistocene glaciation. As far as can be judged from the material available, the 28 chromosome race on the other hand does not extend much outside of the limits of the maximum glaciation. The 28 chromosome race has evidently a much better colonizing ability than the 14 chromosome one because it alone has been able to occupy the glaciated areas in Europe. This view is furthermore supported by the fact that the 28 chromosome race is apparently the only one introduced to North America and New Zealand. In the very scanty Asiatic material the 28 chromosome race is not represented at all. Instead, the 14 chromosome race is found in Siberia even north of the arctic circle, again, however, very close to unglaciated areas.

The karyotypes of 13 strains are analyzed and the karyotype evolution is discussed. Apparently the most primitive race was a 14 chromosome one. By a centric fusion type of interchange it gave rise to a race with a long metacentric and a very small genetically more or less inert chromosome instead of two long subacrocentric ones. Another smaller interchange or a pericentric inversion changed the shape of another chromosome. With loss of the small genetically inert chromosome a 12 chromosome race thus arose. The 28 chromosome race evolved by polyploidization of the 14 chromosome race. *Vicia tenuifolia* probably evolved roughly in the same way from the 12 chromosome race of *Vicia cracca* or from some other closely related 12 chromosome form with the same karyotype. Although the study of the karyotypes of these two tetraploids does not present any definite evidence about the type of polyploidy concerned, neither strict autopolyploidy nor strict allopolyploidy seems plausible in these cases.

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QUANTITATIVE INHERITANCE AND DOMINANCE

II. THE GENIC AND THE DOMINANCE VARIANCE UNDER INBREEDING

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I. INTRODUCTION

THE basis of the partitioning of the genetic variance into an additively genetic (here called genic) and a dominance component was laid by FISHER (1918). The epistatic component was also introduced, but its treatment was rather limited. However, recent work by COCKERHAM (1954) and KEMPTHORNE (1954, 1955 a and b) have lead to new possibilities as regards the epistatic component.

In the light of these new findings it had here been intended to give a full account of the changes that the three genetic variance components undergo under inbreeding. However, mainly due to the fact that the number of subcomponents into which the epistatic component can be divided depends upon the number of loci involved, this study will be limited essentially to the behaviour of the genic and the dominance component when epistasis is not present. Only a few results will be indicated with epistasis present.

It has previously been shown how the total genic variance increases with inbreeding (see *e.g.* LUSH, 1945; LERNER, 1958), when no dominance or epistasis is present. COCKERHAM (1954) included genic effects and dominance as well as epistasis but his treatment was restricted to two alleles at each locus. A study covering only the genic effects and dominance but with any number of alleles at the individual loci therefore seemed justifiable.

II. THE GENIC VARIANCE

Consider a trait that is influenced by n loci, A, B, ..., N. Suppose further that there are m_A alleles at locus A, m_B at locus B, etc., and

that the gene frequencies at locus A are $p_1, p_2, \ldots, p_{m_A}$; at locus B $q_1, q_2, \ldots, q_{m_B}$; ...; and at locus N $w_1, w_2, \ldots, w_{m_N}$. Suppose further that the population is inbred and has reached an inbreeding coefficient of F (WRIGHT, 1922; MALÉCOT, 1948). No mutations are assumed to have occurred during the inbreeding. The genetic value of the genotype $A_iA_jB_kB_l \ldots N_sN_t$ will be denoted by $y^{(F)}_{A_iA_jB_kB_l \ldots N_sN_t}$ and the genetic value of the genotype A_iA_j by $\bar{y}^{(F)}_{A_iA_j}$, the bar over y indicating that averaging has taken place over the loci B, ..., N. The following genetic model will be used:

$$\bar{y}^{(F)}_{A_iA_j} = \alpha_i^{(F)} + \alpha_j^{(F)} + a_{ij}^{(F)} + \mu^{(F)},$$

where $\alpha_i^{(F)}$ = the effect of the gene A_i, determined by the least-square method, etc.,

$a_{ij}^{(F)}$ = the dominance deviation of the genotype A_iA_j, determined as the difference $\bar{y}^{(F)}_{A_iA_j} - \alpha_i^{(F)} - \alpha_j^{(F)} - \mu^{(F)}$,

$\mu^{(F)}$ = the overall mean of the population.

The function, Q, to be minimized in order to find the α's is then

$$Q = \sum_{i=1}^{m_A} [(p_i)^2 + F\, p_i\,(1-p_i)]\left[\bar{y}^{(F)}_{A_iA_i} - 2\,\alpha_i^{(F)} - \mu^{(F)}\right]^2 +$$

$$\sum_{i=1}^{m_A-1} \sum_{\substack{j=2 \\ i<j}}^{m_A} 2\,(1-F)\, p_i p_j \left[\bar{y}^{(F)}_{A_iA_j} - \alpha_i^{(F)} - \alpha_j^{(F)} - \mu^{(F)}\right]^2 =$$

$$(1-F) \sum_{i=1}^{mA} \sum_{j=1}^{m_A} p_i\, p_j \left[\bar{y}^{(F)}_{A_iA_j} - \alpha_i^{(F)} - \alpha_j^{(F)} - \mu^{(F)}\right]^2 +$$

$$F \sum_{i=1}^{m_A} p_i \left[\bar{y}^{(F)}_{A_iA_i} - 2\,\alpha_i^{(F)} - \mu^{(F)}\right]^2.$$

Then $\dfrac{\delta Q}{\delta\, \alpha_{i'}^{(F)}} = 0$ leads to

$$2\,(1-F) \sum_{j=1}^{m_A} p_{i'}\, p_j \left[\bar{y}^{(F)}_{A_{i'}A_j} - \alpha_{i'}^{(F)} - \alpha_j^{(F)} - \mu^{(F)}\right](-1) +$$

$$2\,(1-F) \sum_{i=1}^{m_A} p_i\, p_{i'} \left[\bar{y}^{(F)}_{A_iA_{i'}} - \alpha_i^{(F)} - \alpha_{i'}^{(F)} - \mu^{(F)}\right](-1) +$$

$$2\,F\, p_{i'} \left[\bar{y}^{(F)}_{A_{i'}A_{i'}} - 2\,\alpha_{i'}^{(F)} - \mu^{(F)}\right](-2) = 0$$

or

$$(1-F)\sum_{j=1}^{m_A} p_j\left[\bar{y}^{(F)}_{A_{i'}A_j}-\alpha^{(F)}_{i'}-\alpha^{(F)}_j-\mu^{(F)}\right]+$$

$$F\left[\bar{y}^{(F)}_{A_{i'}A_{i'}}-2\,\alpha^{(F)}_{i'}-\mu^{(F)}\right]=0.$$

Writing i instead of i′ it can be seen that

$$\alpha^{(F)}_i=\frac{(1-F)\sum_{j=1}^{m_A} p_j\,\bar{y}^{(F)}_{A_iA_j}+F\,\bar{y}^{(F)}_{A_iA_i}-\mu^{(F)}}{1+F}, \tag{1 a}$$

as $\sum_{j=1}^{m_A} p_j\,\alpha^{(F)}_j=0$ due to the fact that, by definition,

$$(1-F)\sum_{i=1}^{m_A}\sum_{j=1}^{m_A} p_i\,p_j\left[\alpha^{(F)}_i+\alpha^{(F)}_j\right]+F\sum_{i=1}^{m_A} p_i\left[2\,\alpha^{(F)}_i\right]=0$$

or

$$2\,(1-F)\sum_{i=1}^{m_A} p_i\,\alpha^{(F)}_i+2\,F\sum_{i=1}^{m_A} p_i\,\alpha^{(F)}_i=0$$

and thus

$$\sum_{i=1}^{m_A} p_i\,\alpha^{(F)}_i=0. \tag{2}$$

When F=0

$$\alpha_i=\sum_{j=1}^{m_A} p_j\,\bar{y}_{A_iA_j}-\mu, \tag{1 b}$$

which is the same formula for a random-mating population as that given by KEMPTHORNE (1957).

The genic variance for the A-locus at an inbreeding coefficient of F can be written as

$$\left[\sigma^{(F)}_{G:A}\right]^2=(1-F)\sum_{i=1}^{m_A}\sum_{j=1}^{m_A} p_i\,p_j\left[\alpha^{(F)}_i+\alpha^{(F)}_j\right]^2+$$

$$F\sum_{i=1}^{m_A} p_i\left[2\,\alpha^{(F)}_i\right]^2.$$

Thus

$$\left[\sigma^{(F)}_{G:A}\right]^2=2\,(1+F)\sum_{i=1}^{m_A} p_i\left[\alpha^{(F)}_i\right]^2 \quad \text{(cf. KEMPTHORNE, 1957).} \tag{3 a}$$

As the different loci are independent with respect to their genic effects, the total genic variance is obtained by summation over all loci.

Up to this point the treatment is the same irrespective of whether epistasis is present or not. However, the genic variance is expressed in terms of the genic parameters of the inbred population at a particular inbreeding level. Comparisons of the genic variances at different inbreeding levels are therefore very difficult. It is therefore to be desired to have the different genic variances expressed in terms of a common set of genetic parameters, preferably those of the initial randomly mated population.

The first step in that direction is to find the relations between the $y^{(F)}$-values and the y-values. Suppose the genetic value of the genotype $A_iA_jB_kB_lN_sN_t$ (for simplicity a trait influenced only by the three loci A, B, and N is considered) in the inbred population is $y^{(F)}_{A_iA_jB_kB_lN_sN_t}$. It is, however, obvious that

$$y^{(F)}_{A_iA_jB_kB_lN_sN_t} = y_{A_iA_jB_kB_lN_sN_t}.$$

Therefore, if the alleles at the different loci are randomly combined,

$$\bar{y}^{(F)}_{A_iA_j} = \sum_{k=1}^{m_B}\left[(q_k)^2 + F\,q_k\,(1-q_k)\right]\sum_{s=1}^{m_N}\left[(w_s)^2 + F w_s\,(1-w_s)\right]\times$$

$$y_{A_iA_jB_kB_kN_sN_s} + \sum_{k=1}^{m_B}\left[(q_k)^2 + F q_k\,(1-q_k)\right]\times$$

$$\sum_{s=1}^{m_N}\sum_{\substack{t=1\\ s\neq t}}^{m_N}(1-F)\,w_s\,w_t\,y_{A_iA_jB_kB_kN_sN_t} +$$

$$\sum_{k=1}^{m_B}\sum_{\substack{l=1\\ k\neq l}}^{m_B}(1-F)\,q_k\,q_l\sum_{s=1}^{m_N}\left[(w_s)^2 + F\,w_s\,(1-w_s)\right]\times$$

$$y_{A_iA_jB_kB_lN_sN_s} + \sum_{k=1}^{m_B}\sum_{\substack{l=1\\ k\neq l}}^{m_B}(1-F)\,q_k\,q_l\times$$

$$\sum_{s=1}^{m_N}\sum_{\substack{t=1\\ s\neq t}}^{m_N}(1-F)\,w_s\,w_t\,y_{A_iA_jB_kB_lN_sN_t} =$$

$$\sum_{k=1}^{m_B}\left[(q_k)^2 + F\,q_k\,(1-q_k)\right]\sum_{s=1}^{m_N}\sum_{t=1}^{m_N}(1-F)\,w_s\,w_t\times$$

$$y_{A_iA_jB_kB_kN_sN_t} + \sum_{k=1}^{m_B}\left[(q_k)^2 + F\,q_k\,(1-q_k)\right]\times$$

$$\sum_{s=1}^{m_N} F\, w_s\, y_{A_iA_jB_kB_kN_sN_s} + \sum_{k=1}^{m_B} \sum_{\substack{l=1 \\ k \neq l}}^{m_B} (1-F)\, q_k\, q_l \times$$

$$\sum_{s=1}^{m_N} \sum_{t=1}^{m_N} (1-F)\, w_s\, w_t\, y_{A_iA_jB_kB_lN_sN_t} +$$

$$\sum_{k=1}^{m_B} \sum_{\substack{l=1 \\ k \neq l}}^{m_B} (1-F)\, q_k\, q_l \sum_{s=1}^{m_N} F\, w_s\, y_{A_iA_jB_kB_lN_sN_s} =$$

$$(1-F)\,(1-F) \sum_{k=1}^{m_B} \sum_{l=1}^{m_B} q_k\, q_l \sum_{s=1}^{m_N} \sum_{t=1}^{m_N} w_s\, w_t \times$$

$$y_{A_iA_jB_kB_lN_sN_t} + F\,(1-F) \sum_{k=1}^{m_B} q_k \sum_{s=1}^{m_N} \sum_{t=1}^{m_N} w_s\, w_t \times$$

$$y_{A_iA_jB_kB_kN_sN_t} + (1-F)\, F \sum_{k=1}^{m_B} \sum_{l=1}^{m_B} q_k\, q_l \sum_{s=1}^{m_N} w_s \times$$

$$y_{A_iA_jB_kB_lN_sN_s} + F \times F \sum_{k=1}^{m_B} q_k \sum_{s=1}^{m_N} w_s\, y_{A_iA_jB_kB_kN_sN_s}.$$

Thus

(4 a) $$\bar{y}^{(F)}_{A_iA_j} = (1-F)^2\, \bar{y}_{A_iA_j} + F\,(1-F) \sum_{k=1}^{m_B} q_k\, \bar{y}_{A_iA_jB_kB_k} +$$

$$F\,(1-F) \sum_{s=1}^{m_N} w_s\, \bar{y}_{A_iA_jN_sN_s} + F^2 \sum_{k=1}^{m_B} q_k \sum_{s=1}^{m_N} w_s \times$$

$$y_{A_iA_jB_kB_kN_sN_s}.$$

Provided now that no epistasis is present the genetic model adopted above can be extended to

$$y_{A_iA_jB_kB_lN_sN_t} = \alpha_i + \alpha_j + a_{ij} + \beta_k + \beta_l + b_{kl} +$$

$$\nu_s + \nu_t + n_{st} + \mu,$$

where, as before, Greek letters denote genic effects and Roman letters dominance deviations. Then

$$\bar{y}^{(F)}_{A_iA_j} = (1-F)^2\,(\alpha_i + \alpha_j + a_{ij} + \mu) +$$

$$F\,(1-F) \sum_{k=1}^{m_B} q_k\,(\alpha_i + \alpha_j + a_{ij} + 2\beta_k + b_{kk} + \mu) +$$

$$F\,(1-F) \sum_{s=1}^{m_N} w_s\,(\alpha_i + \alpha_j + a_{ij} + 2\nu_s + n_{ss} + \mu) +$$

$$F^2 \sum_{k=1}^{m_B} q_k \sum_{s=1}^{m_N} w_s\,(\alpha_i + \alpha_j + a_{ij} + 2\beta_k + b_{kk} +$$

$$2\,\nu_s + n_{ss} + \mu).$$

Thus

$$(4\text{ b}) \qquad \bar{y}^{(F)}_{A_iA_j} = \alpha_i + \alpha_j + a_{ij} + F \sum_{k=1}^{m_B} q_k\,b_{kk} + F \sum_{s=1}^{m_N} w_s\,n_{ss} + \mu.$$

Obviously,

$$\bar{y}^{(F)}_{A_iA_i} = 2\,\alpha_i + a_{ii} + F \sum_{k=1}^{m_B} q_k\,b_{kk} + F \sum_{s=1}^{m_N} w_s\,n_{ss} + \mu,$$

and

$$\mu^{(F)} = (1-F) \sum_{i=1}^{m_A} \sum_{j=1}^{m_A} p_i\,p_j\,(\alpha_i + \alpha_j + a_{ij} + F \sum_{k=1}^{m_B} q_k\,b_{kk} +$$

$$F \sum_{s=1}^{m_N} w_s\,n_{ss} + \mu) +$$

$$F \sum_{i=1}^{m_A} p_i\,(2\,\alpha_i + a_{ii} + F \sum_{k=1}^{m_B} q_k\,b_{kk} + F \sum_{s=1}^{m_N} w_s\,n_{ss} + \mu),$$

and, as by definition $\sum_{i=1}^{m_A} \sum_{j=1}^{m_A} p_i\,p_j\,a_{ij} = 0$, thus

$$(5) \qquad \mu^{(F)} = F \sum_{i=1}^{m_A} p_i\,a_{ii} + F \sum_{k=1}^{m_B} q_k\,b_{kk} + F \sum_{s=1}^{m_N} w_s\,n_{ss} + \mu.$$

The expression for $\mu^{(F)}$ in formula 5 is identical with that which KEMPTHORNE (1957) obtained, epistasis excluded.

Formulas 4 a, 4 b, and 5 can be extended to cover any number of loci.

It can now be seen that

$$\alpha_i^{(F)} = \frac{(1+F)\,\alpha_i + F\,a_{ii} - F\sum_{j=1}^{m_A} p_j\,a_{jj}}{1+F} =$$

$$\alpha_i + \frac{F}{1+F}\left(a_{ii} - \sum_{j=1}^{m_A} p_j\,a_{jj}\right), \tag{1 c}$$

as

$$\sum_{j=1}^{m_A} p_j\,a_{jj} = 0.$$

Insertion of the value of $\alpha_i^{(F)}$ in the formula (3 a) for the genic variance at the A-locus leads to

$$\left[\sigma_{G:A}^{(F)}\right]^2 = 2\,(1+F)\sum_{i=1}^{m_A} p_i\left[(\alpha_i)^2 + \frac{F^2}{(1+F)^2}(a_{ii})^2 + \right.$$

$$\frac{F^2}{(1+F)^2}\left(\sum_{j=1}^{m_A} p_j\,a_{jj}\right)^2 + \frac{2F}{1+F}\alpha_i\,a_{ii} -$$

$$\left.\frac{2F}{1+F}\alpha_i\sum_{j=1}^{m_A} p_j\,a_{jj} - \frac{2F^2}{(1+F)^2}a_{ii}\sum_{j=1}^{m_A} p_j\,a_{jj}\right].$$

Thus

$$\left[\sigma_{G:A}^{(F)}\right]^2 = 2\,(1+F)\sum_{i=1}^{m_A} p_i\,(\alpha_i)^2 + 2\,\frac{F^2}{1+F}\sum_{i=1}^{m_A} p_i\,(a_{ii})^2 - \tag{3 b}$$

$$2\,\frac{F^2}{1+F}\left(\sum_{i=1}^{m_A} p_i\,a_{ii}\right)^2 + 4\,F\sum_{i=1}^{m_A} p_i\,\alpha_i\,a_{ii}.$$

Summation over all loci gives the total genic variance, $[\sigma_G^{(F)}]^2$.

With epistasis present the algebra becomes even more tedious. If only two loci, A and B, are considered, the genetic model becomes

$$y_{A_iA_jB_kB_l} = \alpha_i + \alpha_j + a_{ij} + \beta_k + \beta_l + b_{kl} + (\alpha\beta)_{ik} +$$

$$(\alpha\beta)_{il} + (\alpha\beta)_{jk} + (\alpha\beta)_{jl} + (\alpha b)_{ikl} + (\alpha b)_{jkl} +$$

$$(a\beta)_{ijk} + (a\beta)_{ijl} + (ab)_{ijkl} + \mu,$$

where $(\alpha\beta)_{ik}$ = genic × genic (also called additive × additive) epistatic deviation associated with the genes A_i and B_k, etc.;

$(\alpha b)_{ikl}$ = genic × dominance (also called additive × dominance) epistatic deviation associated with the gene A_i and the genotype B_kB_l, etc.;

$(ab)_{ijkl}$ = dominance × dominance epistatic deviation associated with the genotypes A_iA_j and B_kB_l.

(Cf. KEMPTHORNE, 1955 a and 1957). Using the same approach as before it can be shown that

$$(1\,d)\qquad \alpha_i^{(F)} = \alpha_i + \frac{F}{1+F}\left[a_{ii} - \sum_{j=1}^{m_A} p_j\, a_{jj}\right] + F \sum_{k=1}^{m_B} q_k\, (\alpha b)_{ikk} +$$

$$\frac{F^2}{1+F}\left[\sum_{k=1}^{m_B} q_k\, (ab)_{iikk} - \sum_{j=1}^{m_A}\sum_{k=1}^{m_B} p_j\, q_k\, (ab)_{jjkk}\right].$$

It is to be noted that of the epistatic contributions no genic × genic ones are included but only the genic × dominance and dominance × dominance and of these only the ones that involve homozygotes.

Spelling out in full the genic variance leads in this case to an expression of thirteen terms, thus an increase of nine from the case with only genic effects and dominance present.

A similar expression, with thirteen terms, would be obtained, if any number of loci were involved and genic effects, dominance and dual epistasis, *i.e.* epistasis involving only two loci, were considered. Most of these terms would, however, be of a rather complex structure.

III. THE DOMINANCE VARIANCE

The dominance deviation associated with the genotype A_iA_j in the inbred population is:

$$(6\,a)\qquad a_{ij}^{(F)} = \bar{y}_{A_iA_j}^{(F)} - \alpha_i^{(F)} - \alpha_j^{(F)} - \mu^{(F)}$$ (cf. p. 112).

All the terms necessary for its estimation in terms of the genetic parameters of the original randomly mated population, when no epistasis is present, have already been evaluated in the previous section (formulas 4 b, 1 c, and 5). Thus, with any number of loci involved,

$$a_{ij}^{(F)} = \left[\alpha_i + \alpha_j + a_{ij} + F \sum_{k=1}^{m_B} q_k\, b_{kk} + \ldots\ldots + \right.$$

$$\left. F \sum_{s=1}^{m_N} w_s\, n_{ss} + \mu\right] - \left[\alpha_i + \frac{F}{1+F}\, a_{ii} - \right.$$

$$\frac{F}{1+F}\sum_{i'=1}^{m_A} p_{i'}\, a_{i'i'}\Big] - \Big[\alpha_j + \frac{F}{1+F}\, a_{jj} -$$

$$\frac{F}{1+F}\sum_{i'=1}^{m_A} p_{i'}\, a_{i'i'}\Big] - \Big[F\sum_{i'=1}^{m_A} p_{i'}\, a_{i'i'} +$$

$$F\sum_{k=1}^{m_B} q_k\, b_{kk} + \ldots\ldots + F\sum_{s=1}^{m_N} w_s\, n_{ss} + \mu\Big]$$

or

(6 b) $$a_{ij}^{(F)} = a_{ij} - \frac{F}{1+F}(a_{ii} + a_{jj}) + \frac{F(1-F)}{1+F}\sum_{i'=1}^{m_A} p_{i'}\, a_{i'i'}.$$

Now the dominance variance at locus A at inbreeding level F

(7 a) $$\left[\sigma_{D:A}^{(F)}\right]^2 = (1-F)\sum_{i=1}^{m_A}\sum_{j=1}^{m_A} p_i\, p_j \left[a_{ij}^{(F)}\right]^2 + F\sum_{i=1}^{m_A} p_i \left[a_{ii}^{(F)}\right]^2.$$

Therefore, expressed in terms of the parameters of the random-mating population, the dominance variance at locus A

$$\left[\sigma_{D:A}^{(F)}\right]^2 = (1-F)\sum_{i=1}^{m_A}\sum_{j=1}^{m_A} p_i\, p_j \left[(a_{ij})^2 + \frac{F^2}{(1+F)^2}(a_{ii})^2 +\right.$$

$$\frac{F^2}{(1+F)^2}(a_{jj})^2 + \frac{F^2(1-F)^2}{(1+F)^2}\left(\sum_{i'=1}^{m_A} p_{i'}\, a_{i'i'}\right)^2 -$$

$$2\frac{F}{1+F}\, a_{ij}\, a_{ii} - 2\frac{F}{1+F}\, a_{ij}\, a_{jj} +$$

$$2\frac{F(1-F)}{1+F}\, a_{ij}\sum_{i'=1}^{m_A} p_{i'}\, a_{i'i'} + 2\frac{F^2}{(1+F)^2}\, a_{ii}\, a_{jj} -$$

$$2\frac{F^2(1-F)}{(1+F)^2}\, a_{ii}\sum_{i'=1}^{m_A} p_{i'}\, a_{i'i'} -$$

$$\left.2\frac{F^2(1-F)}{(1+F)^2}\, a_{jj}\sum_{i'=1}^{m_A} p_{i'}\, a_{i'i'}\right] +$$

$$F\sum_{i=1}^{m_A} p_i\left[\frac{(1-F)^2}{(1+F)^2}(a_{ii})^2 + \frac{F^2(1-F)^2}{(1+F)^2}\left(\sum_{j=1}^{m_A} p_j\, a_{jj}\right)^2 +\right.$$

$$\left.2\frac{F(1-F)^2}{(1+F)^2}\, a_{ii}\sum_{j=1}^{m_A} p_j\, a_{jj}\right]$$

or

$$(7\text{ b})\quad \left[\sigma^{(F)}_{D:A}\right]^2 = (1-F)\sum_{i=1}^{m_A}\sum_{j=1}^{m_A} p_i\, p_j\, (a_{ij})^2 + \frac{F\,(1-F)}{1+F}\sum_{i=1}^{m_A} p_i\, (a_{ii})^2 + \frac{F^2\,(1-F)}{1+F}\left(\sum_{i=1}^{m_A} p_i\, a_{ii}\right)^2.$$

The total dominance variance is, of course, obtained by summation over loci.

Comparison of the dominance variance with the genic variance (formula 3 b) shows that the genetic quantities $\sum_{i=1}^{m_A} p_i\, (a_{ii})^2$ and $\left(\sum_{i=1}^{m_A} p_i\, a_{ii}\right)^2$ occur in both variance components. Only one new genetic quantity, $\sum_{i=1}^{m_A}\sum_{j=1}^{m_A} p_i\, p_j\, (a_{ij})^2$ or the dominance variance of the original randomly mated population, is therefore introduced. The inbreeding coefficient and five genetic quantities involving parameters of the original random-mating population are thus sufficient to describe the changes that the genic and the dominance variance undergo under inbreeding, when no epistasis is present, and when any number of loci and any number of alleles at each locus are involved.

When there are only two alleles at each locus the number of genetic quantities is reduced to four, as in this case $\left(\sum_{i=1}^{m_A} p_i\, a_{ii}\right)^2 = \sum_{i=1}^{m_A}\sum_{j=1}^{m_A} p_i\, p_j\, (a_{ij})^2$. The proof of this is as follows:

As

$$\sum_{j=1}^{m_A} p_j\, a_{ij} = 0,$$

$$p_1\, a_{11} + p_2\, a_{12} = 0$$

and

$$p_1\, a_{12} + p_2\, a_{22} = 0$$

or

$$a_{11} = -\frac{p_2}{p_1}\, a_{12}$$

and

$$a_{22} = -\frac{p_1}{p_2}\, a_{12}.$$

Therefore

$$\left(\sum_{i=1}^{m_A} p_i\, a_{ii}\right)^2 = (p_1\, a_{11} + p_2\, a_{22})^2 = (p_1)^2\,(a_{11})^2 +$$

$$2\, p_1\, p_2\, a_{11}\, a_{22} + (p_2)^2\,(a_{22})^2 =$$

$$(p_1)^2\,(a_{11})^2 + 2\, p_1\, p_2\,(a_{12})^2 + (p_2)^2\,(a_{22})^2$$

and thus

$$\left(\sum_{i=1}^{m_A} p_i\, a_{ii}\right)^2 = \sum_{i=1}^{m_A}\sum_{j=1}^{m_A} p_i\, p_j\,(a_{ij})^2. \tag{8}$$

When epistasis is present and the two loci A and B are considered, it can be shown that

$$a_{ij}^{(F)} = a_{ij} - \frac{F}{1+F}(a_{ii} + a_{jj}) + \frac{F\,(1-F)}{1+F}\sum_{i'=1}^{m_A} p_{i'}\, a_{i'i'} + \tag{6 c}$$

$$F\sum_{k=1}^{m_B} q_k\,(ab)_{ijkk} - \frac{F^2}{1+F}\left[\sum_{k=1}^{m_B} q_k\,(ab)_{iikk} + \right.$$

$$\left.\sum_{k=1}^{m_B} q_k\,(ab)_{jjkk}\right] + \frac{F^2\,(1-F)}{1+F}\sum_{i'=1}^{m_A}\sum_{k=1}^{m_B} p_{i'}\, q_k\,(ab)_{i'i'kk}.$$

It is thus evident that only dominance and dominance × dominance deviations are involved.

The dominance variance is now a function of the inbreeding coefficient and nine genetic quantities which are all functions of the genetic parameters of the original randomly mated population. Of these nine quantities six appear in the corresponding genic variance. The values of sixteen different genetic quantities must therefore be known in this case before the relationships can be established between the inbreeding level on one side and the genic and the dominanc variance on the other side.

IV. EXAMPLES

A few examples will be given in order to give a more vivid illustration of the changes that the genic and the dominance variance undergo under inbreeding. For simplicity only one-locus cases will be treated.

The first example, Fig. 1, consists of one locus with two alleles, each with a gene frequency of 0.5. Let the locus be A, the alleles A_1 and A_2. The homozygote A_1A_1 has been assigned a genetic value of 0, the homo-

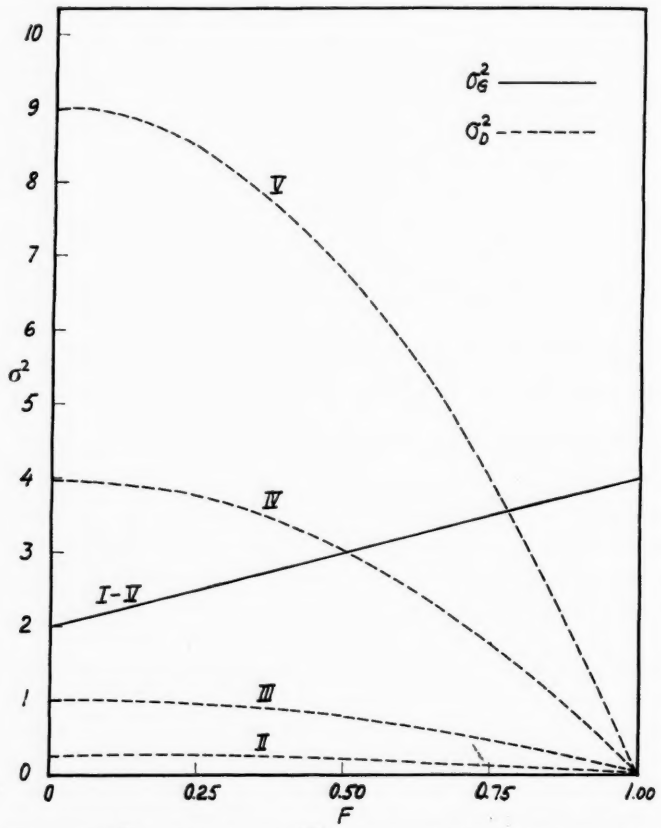

Fig. 1. The genic (σ_G^2) and dominance (σ_D^2) variance at different degrees of inbreeding (F) and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, two alleles, gene frequencies 0.5. For further details, see text!

zygote A_2A_2 a value of 4. The heterozygote, A_1A_2, assumes different values: 2 (no dominance), 3 (partial dominance), 4 (complete dominance), 6 (overdominance, case 1), and 8 (overdominance, case 2). As in this case $a_{11}=a_{22}$ (cf. p. 120) and therefore $a_{ii}=\sum_{j=1}^{m_A} p_j a_{jj}$ and thus $\alpha_i^{(F)}=\alpha_i$ (see formula 1 c), the genic variance is independent of the degree of dominance and increases linearly with $(1+F)$. The magnitude of the dominance variance increases considerably with the degree of

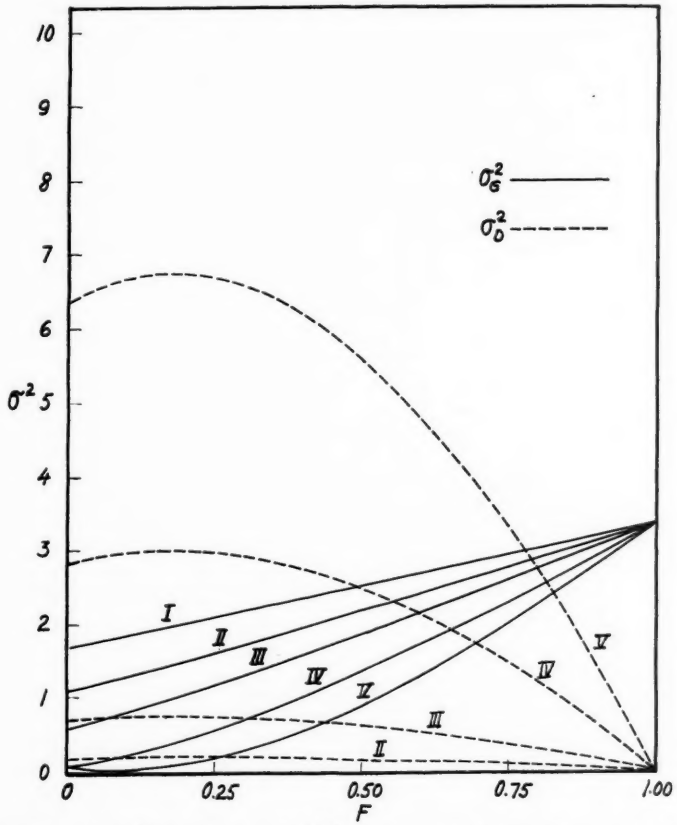

Fig. 2. The genic $\left(\sigma_G^2\right)$ and dominance $\left(\sigma_D^2\right)$ variance at different degrees of inbreeding (F) and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, two alleles, gene frequencies 0.3 and 0.7. For further details, see text!

dominance. For each degree of dominance, the dominance variance is largest, when F=0.

The example in Fig. 2 is the same as that in Fig. 1 with the exception that the frequency of gene A_1 is 0.3 and that of gene A_2 0.7. As expected the magnitude of the variances is now decreased. With increasing degree of dominance the genic variance in this case becomes more and more curvi-linear; at the highest degree of dominance the genic variance has even a minimum, at which it is =0. The dominance variance,

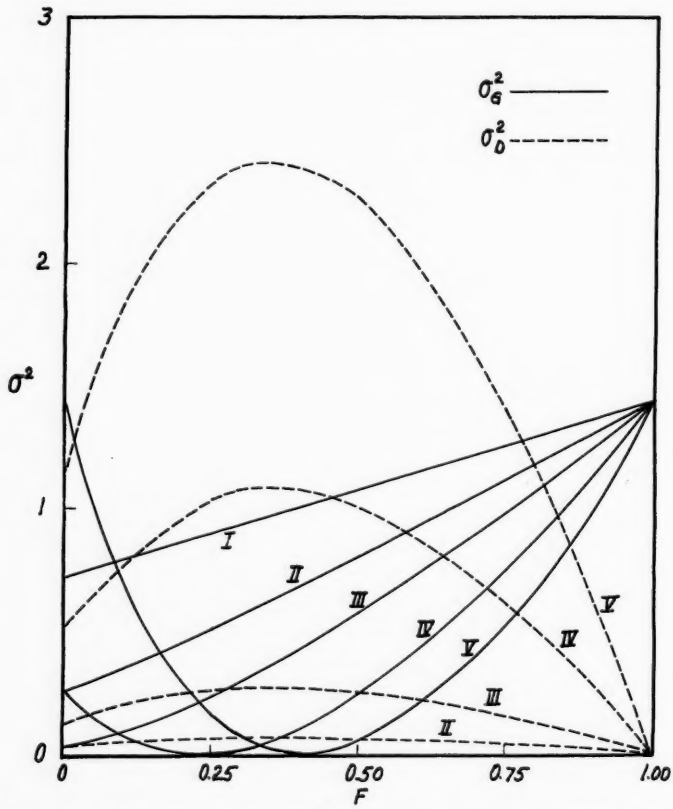

Fig. 3. The genic (σ^2_G) and dominance (σ^2_D) variance at different degrees of inbreeding (F) and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, two alleles, gene frequencies 0.1 and 0.9. For further details, se text! Note that a different scale is used here than in Figs. 1 and 2!

on the other hand, shows a maximum value (at F=0.17) for all degrees of dominance.

In Fig. 3 the gene frequencies have been further changed, to 0.1 for the allele A_1 and to 0.9 for A_2. The curvi-linearity of the genic variance is here even more pronounced. Both cases of overdominance now have a minimum (at which the genic variance =0). The dominance variance shows, as in Fig. 2, a maximum value (at F=0.35 for all degrees of dominance).

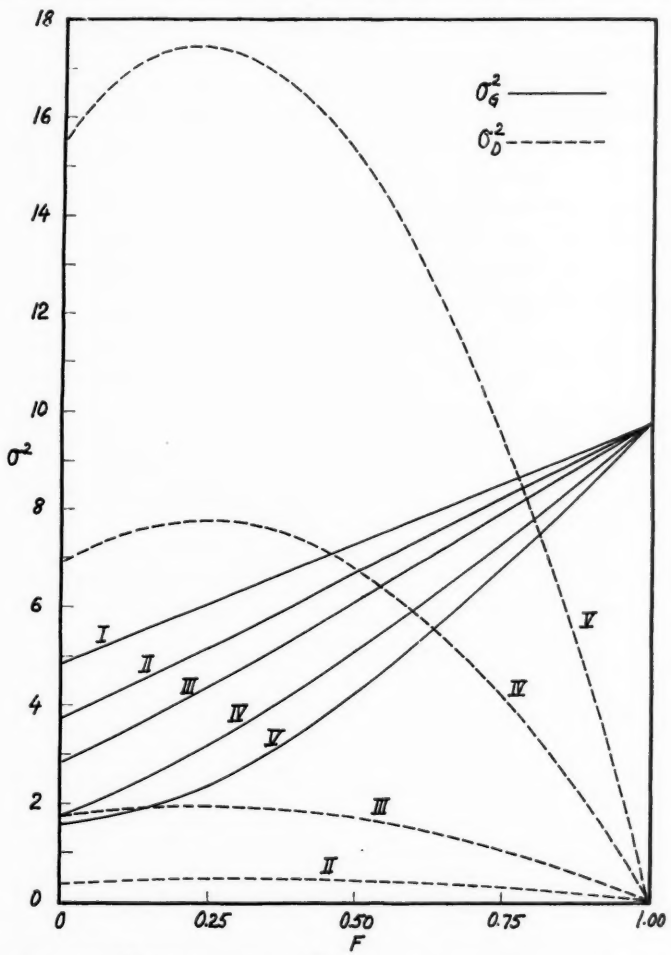

Fig. 4. The genic $\left(\sigma^2_G\right)$ and dominance $\left(\sigma^2_D\right)$ variance at different degrees of inbreeding (F) and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, three alleles, gene frequencies 0.2, 0.3 and 0.5, the same degree of dominance exhibited by all heterozygotes. For further details, see text!

Fig. 4 shows a case with three alleles, A_1, A_2 and A_3. The frequency of A_1 is 0.2, of A_2 0.3, and of A_3 0.5. The genetic value of A_1A_1 is 0, of A_2A_2 4, and of A_3A_3 8. The genetic values of A_1A_2, A_1A_3 and A_2A_3 are,

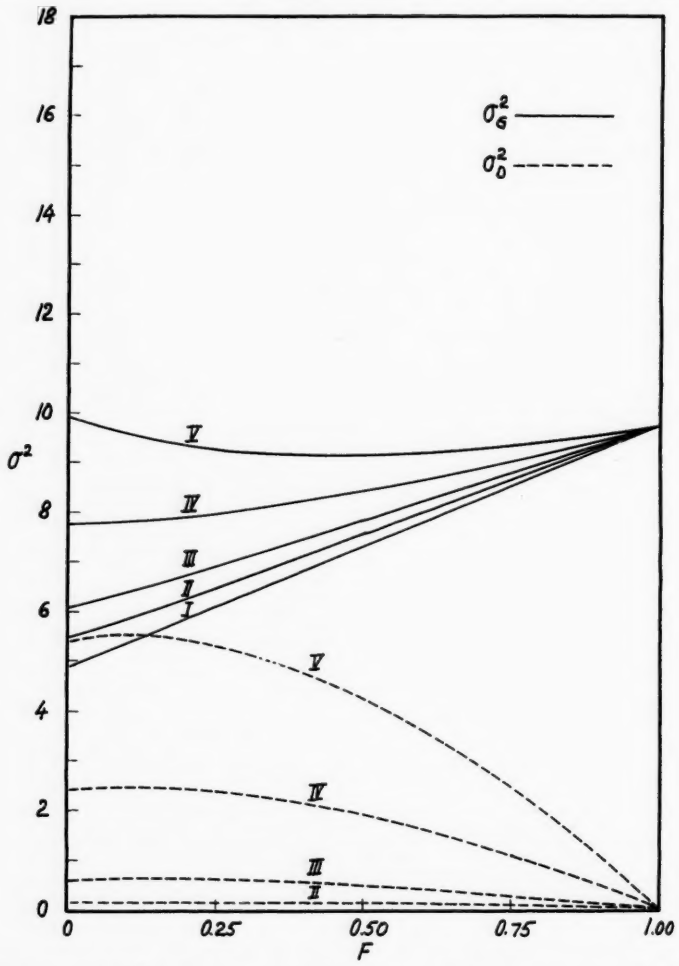

Fig. 5. The genic (σ^2_G) and dominance (σ^2_D) variance at different degrees of inbreeding (F) and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, three alleles, gene frequencies 0.2, 0.3 and 0.5, dominance exhibited only by the heterozygote of the two alleles with the highest gene frequencies. For further details, see text!

respectively, for different degrees of dominance: 2, 4, 6 (no dominance); 3, 6, 7 (partial dominance); 4, 8, 8 (complete dominance); 6, 12, 10 (overdominance, case 1); and 8, 16, 12 (overdominance, case 2). The

behaviour of the genic variance is similar to that of the two-allele case with gene frequencies 0.3 and 0.7 (Fig. 2) with the exception that no minimum occurs at the highest degree of overdominance. The dominance variance also shows most similarity to the same two-allele case (Fig. 2) (the maximi of the dominance variances occur this time at F=0.23).

In Fig. 5 dominance is exhibited only by the heterozygote A_2A_3, otherwise this example is identical with the previous one. The dominance variances behave pretty much in the same way as in Fig. 4 (maximi now at F=0.10), but the values are, of course, much lower than before. The genic variances, on the other hand, behave rather unexpectedly. Now, at each inbreeding level, the genic variance increases with increasing degree of dominance (except at F=1).

V. DISCUSSION

From the formulas given above for the genic variance in inbred populations it is evident that when no dominance or epistasis is present the genic variance increases as 1+F as the inbreeding level, F, increases, thus in accordance with *e.g.* LUSH (1945), LERNER (1958). This is also true when dominance is present and, for each locus, the dominance deviations of the homozygotes are the same ($a_{ii}=\sum_{j=1}^{m_A} p_j a_{jj}$, formula 1 c); this occurs in the case of two alleles at each locus, when the gene frequencies are 0.5 (see p. 120) (cf. KEMPTHORNE, 1957). From formula 1 d it is also clear that the factor 1+F holds, if epistasis of the genic×genic type is present, probably also if epistasis of the types genic×genic×genic, etc., is present. This simple relationship between genic variance and inbreeding level also exists under some circumstances, when other types of epistasis are present. However, these circumstances must, at least for the time being, be regarded as very special cases. The general rule therefore becomes: the increase in genic variance with inbreeding is proportional to 1+F if only genic, genic× genic, etc., variance are present and if, with dominance variance present, at each locus the dominance deviations of the homozygotes are all the same. As can be seen from the Figs. 1—5, it is very difficult to estimate the bias that would exist, if the factor 1+F was used and the requirements just stated were not fulfilled. No attempt has therefore been made to estimate this bias under different conditions. However, in general, the higher the degree of dominance is, the larger is the bias,

and the more the gene frequencies depart from 0.5, the larger is the bias.

It must perhaps be emphasized that the conclusions above are true only, if the inbreeding starts with a population that has been randomly mated for some time, so that the different loci and the different alleles are randomly combined (the coefficients of departure from random combination are all $=1$, KIMURA, 1958).

The dominance variance decreases with inbreeding in a curvi-linear manner or increases at first to a maximum and then decreases, when genic, dominance and epistatic genic$\times$genic and genic$\times$dominance variance are present (cf. formula 6 c and Figs. 1—5). Probably the only epistatic types to be excluded are dominance$\times$dominance, dominance$\times$dominance$\times$dominance, etc. When $F=1$, the dominance variance is in all cases $=0$. The magnitude of the dominance variance increases with the degree of dominance. When dominance is only partial the dominance variance is almost negligible in most instances, when overdominance prevails the dominance variance may be far larger than the genic variance.

It was shown above that when epistasis is not present the inbreeding coefficient and five genetic quantities expressed in parameters of the original random-mating population are sufficient to describe the genic and the dominance variance under inbreeding. Two of these quantities were the genic variance and the dominance variance of the random mating population. Two others were functions of the gene frequencies and the dominance deviations of the homozygotes; they bear some resemblance to the term involving dominance deviations in the formula for the mean of an inbred population given first by KEMPTHORNE (1957) and discussed above (formula 5). The fifth quantity includes the genic effects as well as the gene frequencies and the dominance deviations of the homozygotes.

When dual epistasis is present as well as genic effects and dominance the number of genetic quantities increases rapidly. However, if only epistasis of the type genic$\times$genic is present, the number of quantities is still five, if only the genic and the dominance variance are to be determined under inbreeding. If now the genic$\times$genic variance is to be estimated, too, fortunately the genic$\times$genic epistatic deviations remain the same under inbreeding as in the random-mating population. The genic$\times$genic variance of an inbred population is therefore a simple function of the value that it assumes in the random-mating population; its increase is proportional to $(1+F)^2$ (cf. COCKERHAM, 1954).

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SUMMARY

Inbreeding brings about changes in zygote frequencies. This means that estimates of genetic parameters in the original random-mating population are not valid in the inbred populations. The relationships that exist between estimates of a few of the most important genetic parameters in a random-mating population and in inbred populations derived from it are studied in this paper.

Formulas for the effects of genes as determined by the least-square method and the dominance deviations are developed for the case, when only genic effects and dominance are present, and for the case, when dual epistasis (epistasis involving two loci) as well as genic effects and dominance are present. These formulas are used to describe the changes that the genic (or additively genetic) variance and the dominance variance undergo under inbreeding, when no epistasis is present. It turns out that the inbreeding coefficient and five genetic quantities expressed in parameters of the original random-mating population are sufficient to describe these changes. Two of these quantities are the genic variance and the dominance variance, two others are functions of the gene frequencies and the dominance deviations of the homozygotes, the fifth quantity is a function of the gene frequencies, the effects of the genes and the dominance deviations of the homozygotes. With epistasis present the number of genetic quantities increases considerably. However, if only epistasis of the type genic×genic (or additive×additive) is present no more quantities are required to describe the changes of the genic and of the dominance variance under inbreeding. The genic×genic epistatic variance itself depends upon the inbreeding coefficient and the genic×genic variance of the random-mating population.

It is discussed under what circumstances the increase of the genic variance is proportional to $1+F$, where F is the inbreeding coefficient as defined by WRIGHT or MALÉCOT.

The changes of the genic variance and of the dominance variance with inbreeding are also shown for five arbitrarily chosen one-locus cases (Figs. 1—5).

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QUANTITATIVE INHERITANCE AND DOMINANCE

III. THE GENETIC COVARIANCE OF RELATIVES IN INBRED POPULATIONS

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I. INTRODUCTION

IN random mating populations the genetic part of the covariance of relatives is a linear function of the genetic variance components (FISHER, 1918; KEMPTHORNE, 1957; LAGERVALL, 1960). The factor attached to the genic (additively genetic) variance equals the coefficient of relationship (WRIGHT, 1921 and 1922) or twice the "coefficient de parenté" (MALÉCOT, 1948) while the factor attached to the dominance variance equals the dominance coefficient of relationship (LAGERVALL, 1960). The factors attached to the different epistatic variance components are multiples of these coefficients. Obviously the factors function as correlation coefficients.

In inbred populations no corresponding relationship between the genetic covariance of relatives and the genetic variance components has been found so far. KEMPTHORNE (1955) studied full-sib mating in this respect and HORNER (1956) a system of parent-offspring mating. Both studies were restricted to one locus with two alleles. Furthermore, comparisons were made only between the total genetic covariance on the one hand and the genic and the dominance variance of the inbred and of the original random-mating population on the other hand. Therefore, if any simple relationship exists, this might have been difficult to detect, as the covariance was not divided into a genic and a dominance fraction. It was, however, noted that, when no dominance is present, the genetic correlation between relatives equals the coefficient of relationship. This was first shown by WRIGHT (1921).

In this paper the genetic covariance of relatives in an inbred popula-

tion will be split into its different genetic components. For simplicity, only one locus will be considered but any number of alleles will be included. As the full-sib mating system has been rather extensively explored (HALDANE, 1937, 1955; FISHER, 1949; KEMPTHORNE, 1955) and these results can be utilized here, this inbreeding system was chosen to exemplify the principles involved.

II. THE COVARIANCE OF FULL SIBS UNDER FULL-SIB MATING

Let the locus studied be the A-locus. Suppose that the A-locus has m alleles, $A_1, A_2, \ldots, A_m$. Assume that corresponding gene frequencies are $p_1, p_2, \ldots, p_m$. If the inbreeding is started with the mating of unrelated individuals (generation 0), the following frequencies of different kinds of matings are, after simplification, obtained in generation n from FISHER (1949) and KEMPTHORNE (1955):

Kind of mating	*Frequency*
$A_iA_i \times A_iA_i$	$p_i - \frac{1}{2} p_i (1-p_i) S_n - \frac{8}{5} p_i (1-p_i) S_{n+1} + p_i (1-p_i) (1-2p_i) \left(\frac{1}{2}\right)^n - \frac{1}{5} p_i (1-p_i) [1-5 p_i (1-p_i)] \left(\frac{1}{4}\right)^n$
$A_iA_i \times A_iA_j$	$\frac{2}{5} p_i p_j S_n + \frac{4}{5} p_i p_j S_{n+1} - 2 p_i p_j (1-2p_i) \left(\frac{1}{2}\right)^n + \frac{4}{5} p_i p_j [1-5 p_i (1-p_i)] \left(\frac{1}{4}\right)^n$
$A_iA_i \times A_jA_j$	$\frac{1}{5} p_i p_j S_n - \frac{2}{5} p_i p_j (1-p_i-p_j) \left(\frac{1}{2}\right)^n + \frac{2}{15} p_i p_j [2-5 (p_i+p_j)+15 p_i p_j] \left(\frac{1}{4}\right)^n - \frac{4}{15} p_i p_j (1-p_i-p_j) \left(-\frac{1}{8}\right)^n$

$$A_iA_i \times A_jA_k \quad \frac{4}{5} p_i\, p_j\, p_k \left(\frac{1}{2}\right)^n - \frac{4}{3} p_i\, p_j\, p_k\, (1 - 3\, p_i) \left(\frac{1}{4}\right)^n + \frac{8}{15} p_i\, p_j\, p_k \left(-\frac{1}{8}\right)^n$$

$$A_iA_j \times A_iA_j \quad \frac{8}{5} p_i\, p_j\, S_{n+1} - \frac{8}{5} p_i\, p_j\, (1 - p_i - p_j) \left(\frac{1}{2}\right)^n + \frac{4}{15} p_i\, p_j\, [2 - 5\,(p_i + p_j) + 15\, p_i\, p_j] \left(\frac{1}{4}\right)^n + \frac{4}{15} p_i\, p_j\, (1 - p_i - p_j) \left(-\frac{1}{8}\right)^n$$

$$A_iA_j \times A_iA_k \quad \frac{16}{5} p_i\, p_j\, p_k \left(\frac{1}{2}\right)^n - \frac{8}{3} p_i\, p_j\, p_k\, (1 - 3\, p_i) \left(\frac{1}{4}\right)^n - \frac{8}{15} p_i\, p_j\, p_k \left(-\frac{1}{8}\right)^n$$

$$A_iA_j \times A_kA_l \quad 8\, p_i\, p_j\, p_k\, p_l \left(\frac{1}{4}\right)^n$$

where $S_n = \varepsilon^n + (\varepsilon')^n$ and $S_{n+1} = \varepsilon^{n+1} + (\varepsilon')^{n+1}$ (KEMPTHORNE, 1955) and $\varepsilon = \frac{1}{4}(1 + \sqrt{5})$ and $\varepsilon' = \frac{1}{4}(1 - \sqrt{5})$ (FISHER, 1949).

The following genetic model will be used (cf. LAGERVALL, 1961):

$$y^{(n)}_{A_iA_j} = \alpha^{(n)}_i + \alpha^{(n)}_j + a^{(n)}_{ij} + \mu^{(n)},$$

where $y^{(n)}_{A_iA_j}$ = the genetic value of the genotype A_iA_j in the n^{th} generation of full sibbing,

$\alpha^{(n)}_i$ = the effect of the gene A_i in generation n, determined by the least-square method, etc.,

$a^{(n)}_{ij}$ = the dominance deviation of the genotype A_iA_j in generation n, determined as the difference

$$y^{(n)}_{A_iA_j} - \alpha^{(n)}_i - \alpha^{(n)}_j - \mu^{(n)},$$

$\mu^{(n)}$ = the overall mean of the n^{th} generation.

$\alpha^{(n)}_i + \alpha^{(n)}_j$ then constitutes the genic value of the genotype A_iA_j.

The genic part of the covariance of full sibs in generation n of full-sib mating can now be written down as this part equals the covariance of the genic values of full sibs in generation n. It will consist of $m\left[\alpha^{(n)}_i\right]^2$-terms and $\frac{m(m-1)}{2}\left[\alpha^{(n)}_i\ \alpha^{(n)}_j\right]$-terms. The frequency of each $\left[\alpha^{(n)}_i\right]^2$-term is:

$$4\ p_i - 2\ p_i\ (1-p_i)\ S_n - \frac{32}{5} p_i\ (1-p_i)\ S_{n+1} +$$

$$4\ p_i\ (1-p_i)\ (1-2p_i)\left(\frac{1}{2}\right)^n -$$

$$\frac{4}{5} p_i\ (1-p_i)\left[1-5\ p_i\ (1-p_i)\right]\left(\frac{1}{4}\right)^n +$$

$$\frac{4}{5} p_i\ S_n \sum_{\substack{j=1\\ j\neq i}}^{m} p_j + \frac{8}{5} p_i\ S_{n+1} \sum_{\substack{j=1\\ j\neq i}}^{m} p_j - 4\ p_i\ (1-2\ p_i)\left(\frac{1}{2}\right)^n \sum_{\substack{j=1\\ j\neq i}}^{m} p_j +$$

$$\frac{8}{5} p_i\left[1-5\ p_i\ (1-p_i)\right]\left(\frac{1}{4}\right)^n \sum_{\substack{j=1\\ j\neq i}}^{m} p_j + \frac{8}{5} p_i\ S_{n+1} \sum_{\substack{j=1\\ j\neq i}}^{m} p_j -$$

$$\frac{8}{5} p_i \left(\frac{1}{2}\right)^n \sum_{\substack{j=1\\ j\neq i}}^{m} p_j\ (1-p_i-p_j) +$$

$$\frac{4}{15} p_i \left(\frac{1}{4}\right)^n \sum_{\substack{j=1\\ j\neq i}}^{m} p_j\left[2-5\ (p_i+p_j)+15\ p_i\ p_j\right] +$$

$$\frac{4}{15} p_i \left(-\frac{1}{8}\right)^n \sum_{\substack{j=1\\ j\neq i}}^{m} p_j\ (1-p_i-p_j) + \frac{16}{5} p_i \left(\frac{1}{2}\right)^n \sum_{\substack{j=1\\ j\neq i}}^{m-1} p_j \sum_{\substack{k=2\\ j<k\\ k\neq i}}^{m} p_k -$$

$$\frac{8}{3} p_i (1 - 3 p_i) \left(\frac{1}{4}\right)^n \sum_{\substack{j=1 \\ j \neq i}}^{m-1} p_j \sum_{\substack{k=2 \\ j<k \\ k \neq i}}^{m} p_k - \frac{8}{15} p_i \left(-\frac{1}{8}\right)^n \sum_{\substack{j=1 \\ j \neq i}}^{m-1} p_j \sum_{\substack{k=2 \\ j<k \\ k \neq i}}^{m} p_k =$$

$$4 p_i - \frac{6}{5} p_i (1 - p_i) S_n - \frac{16}{5} p_i (1 - p_i) S_{n+1}.$$

In the same way the frequency of each $\left[\alpha_i^{(n)} \alpha_j^{(n)}\right]$-term =

$$\frac{12}{5} p_i p_j S_n + \frac{32}{5} p_i p_j S_{n+1}.$$

The genic covariance of full sibs in generation n is thus for the A-locus

$$\mathrm{Cov}_{G:A}(\text{F. S. gen. n}) = 4 \sum_{i=1}^{m} p_i \left[\alpha_i^{(n)}\right]^2 -$$

$$\frac{6}{5} S_n \sum_{i=1}^{m} p_i \left[\alpha_i^{(n)}\right]^2 + \frac{6}{5} S_n \sum_{i=1}^{m} (p_i)^2 \left[\alpha_i^{(n)}\right]^2 -$$

$$\frac{16}{5} S_{n+1} \sum_{i=1}^{m} p_i \left[\alpha_i^{(n)}\right]^2 + \frac{16}{5} S_{n+1} \sum_{i=1}^{m} (p_i)^2 \left[\alpha_i^{(n)}\right]^2 +$$

$$\frac{6}{5} S_n \sum_{i=1}^{m} p_i \alpha_i^{(n)} \sum_{\substack{j=1 \\ j \neq i}}^{m} p_j \alpha_j^{(n)} +$$

$$\frac{16}{5} S_{n+1} \sum_{i=1}^{m} p_i \alpha_i^{(n)} \sum_{\substack{j=1 \\ j \neq i}}^{m} p_j \alpha_j^{(n)}.$$

Thus

$$\text{(1 a)} \quad \mathrm{Cov}_{G:A}(\text{F. S. gen. n}) = \left(4 - \frac{6}{5} S_n - \frac{16}{5} S_{n+1}\right) \sum_{i=1}^{m} p_i \left[\alpha_i^{(n)}\right]^2,$$

as

$$\sum_{i=1}^{m} (p_i)^2 \left[\alpha_i^{(n)}\right]^2 + \sum_{i=1}^{m} p_i \alpha_i^{(n)} \sum_{\substack{j=1 \\ j \neq i}}^{m} p_j \alpha_j^{(n)} = \left[\sum_{i=1}^{m} p_i \alpha_i^{(n)}\right]^2$$

and

$$\sum_{i=1}^{m} p_i \alpha_i^{(n)} = 0 \text{ (LAGERVALL, 1961)}.$$

Now

$$S_n = \frac{1}{2} + F_n - \frac{3}{2} F_{n-1}$$

and

$$S_{n+1} = \frac{3}{4} - F_n + \frac{1}{4} F_{n-1} \text{ (KEMPTHORNE, 1955),}$$

where F_n = the inbreeding coefficient (WRIGHT, 1921 and 1922) in the n^{th} generation, etc.

The genic covariance of full sibs in generation n of brother-sister mating can thus, as regards locus A, be written as,

$$\text{(1 b)} \quad \text{Cov}_{G:A}(\text{F. S. gen. n}) = 2 \left(\frac{1}{2} + F_n + \frac{1}{2} F_{n-1} \right) \sum_{i=1}^{m} p_i \left[\alpha_i^{(n)} \right]^2.$$

Division of this covariance by the genic variance of generation n for locus A

$$\left[\sigma_{G:A}^{(n)} \right]^2 = 2 \, (1 + F_n) \sum_{i=1}^{m} p_i \left[\alpha_i^{(n)} \right]^2 \text{ (LAGERVALL, 1961)}$$

gives the correlation between the genic values of full sibs in generation n or the genic correlation of full sibs in generation n

$$\text{(2)} \qquad r_{\text{F. S. gen. n}}^{G} = \frac{\frac{1}{2} + F_n + \frac{1}{2} F_{n-1}}{1 + F_n},$$

which, as expected, is the same as the coefficient of relationship between full sibs in generation n of brother-sister mating (WRIGHT, 1921 and 1922).

The relations between the $\alpha_i^{(n)}$'s and the genetic parameters of the original random-mating population were dealt with in an earlier paper (LAGERVALL, 1961). With no epistasis (or with epistasis of only the type genic×genic) present the genic covariance of full sibs in generation n can, for locus A, be written as

$$\text{(1 c)} \qquad \text{Cov}_{G:A}(\text{F. S. gen. n}) = (1 + 2 F_n + F_{n-1}) G_A +$$

$$\frac{(F_n)^2 (1 + 2 F_n + F_{n-1})}{(1 + F_n)^2} (D'_A - D''_A) +$$

$$\frac{2 F_n (1 + 2 F_n + F_{n-1})}{1 + F_n} C_A,$$

where $G_A = \sum_{i=1}^{m} p_i (\alpha_i)^2$,

$$D'_A = \sum_{i=1}^{m} p_i (a_{ii})^2,$$

$$D''_A = \left(\sum_{i=1}^{m} p_i a_{ii}\right)^2, \text{ and}$$

$$C_A = \sum_{i=1}^{m} p_i \alpha_i a_{ii}.$$

Application of the same technique to the dominance deviations as was done to the genic values leads to the following dominance covariance of full sibs in generation n (locus A):

(3 a) $\mathrm{Cov}_{D:A}$(F. S. gen. n) =

$$\left[\frac{3}{5} - \frac{4}{5}F_n + \frac{1}{5}F_{n-1} - \frac{4}{5}\left(\frac{1}{2}\right)^n + \frac{4}{15}\left(\frac{1}{4}\right)^n + \frac{2}{15}\left(-\frac{1}{8}\right)^n\right] \times$$

$$\sum_{i=1}^{m}\sum_{j=1}^{m} p_i p_j \left[a_{ij}^{(n)}\right]^2 +$$

$$\left[-\frac{9}{20} + \frac{11}{10}F_n + \frac{7}{20}F_{n-1} + \left(\frac{1}{2}\right)^n - \frac{1}{5}\left(\frac{1}{4}\right)^n\right] \sum_{i=1}^{m} p_i \left[a_{ii}^{(n)}\right]^2 +$$

$$\left[\frac{1}{20} + \frac{1}{10}F_n - \frac{3}{20}F_{n-1} - \frac{1}{5}\left(\frac{1}{2}\right)^n + \frac{2}{15}\left(\frac{1}{4}\right)^n - \frac{2}{15}\left(-\frac{1}{8}\right)^n\right] \times$$

$$\left[\sum_{i=1}^{m} p_i a_{ii}^{(n)}\right]^2 + \left[\frac{4}{5} - \frac{2}{5}F_n - \frac{2}{5}F_{n-1} - 2\left(\frac{1}{2}\right)^n + \frac{4}{5}\left(\frac{1}{4}\right)^n\right] \times$$

$$\sum_{i=1}^{m} p_i a_{ii}^{(n)} \sum_{j=1}^{m} p_j a_{ij}^{(n)} +$$

$$\left[\frac{8}{5}\left(\frac{1}{2}\right)^n - \frac{4}{3}\left(\frac{1}{4}\right)^n - \frac{4}{15}\left(-\frac{1}{8}\right)^n\right] \sum_{i=1}^{m} p_i \left[\sum_{j=1}^{m} p_j a_{ij}^{(n)}\right]^2 +$$

$$\left[\frac{2}{5}\left(\frac{1}{2}\right)^n - \frac{2}{3}\left(\frac{1}{4}\right)^n + \frac{4}{15}\left(-\frac{1}{8}\right)^n\right] \times$$

$$\sum_{i=1}^{m} p_i \, a_{ii}^{(n)} \sum_{j=1}^{m} \sum_{k=1}^{m} p_j \, p_k \, a_{jk}^{(n)} +$$

$$\left(\frac{1}{4}\right)^n \left[\sum_{i=1}^{m} \sum_{j=1}^{m} p_i \, p_j \, a_{ij}^{(n)}\right]^2.$$

Unfortunately, it does not seem possible to simplify this rather complex covariance any further. As to its correctness, it can be seen that under random mating (n=1) the covariance of full sibs due to dominance

(3 b) $\quad \mathrm{Cov}_{D:A}(\text{F. S. gen. 1}) = \frac{1}{4} \sum_{i=1}^{m} \sum_{j=1}^{m} p_i \, p_j \left[a_{ij}^{(1)}\right]^2.$

As the dominance variance under random mating

$$(\sigma_{D:A})^2 = \sum_{i=1}^{m} \sum_{j=1}^{m} p_i \, p_j \left[a_{ij}^{(1)}\right]^2,$$

the correlation between the dominance deviations of full sibs under random mating

(4) $\quad r^{D}_{\text{F. S. gen. 1}} = \frac{1}{4}$ (cf. FISHER, 1918).

This correlation thus equals the coefficient of relationship caused by dominance (LAGERVALL, 1960).

The dominance variance in generation n

$$\left[\sigma_{D:A}^{(n)}\right]^2 = (1 - F_n) \sum_{i=1}^{m} \sum_{j=1}^{m} p_i \, p_j \left[a_{ij}^{(n)}\right]^2 + F_n \sum_{i=1}^{m} p_i \left[a_{ii}^{(n)}\right]^2$$

(LAGERVALL, 1961). It is thus obvious that in any inbred generation the correlation between the dominance deviations of full sibs does not equal the dominance coefficient of relationship between full sibs (LAGERVALL, 1960).

It is to be noted that all the latent roots of the generation matrix are included in the dominance covariance and thus not only the roots 1, ε, ε', as was the case for the genic covariance.

If now no epistasis (or epistasis of only the type genic×genic, cf. LAGERVALL, 1961) is present, the dominance covariance in generation n can, as regards locus A, be written as:

(3 b) $\quad \mathrm{Cov}_{D:A}(\text{F. S. gen. n}) =$

$$\left[\frac{3}{5}-\frac{4}{5}F_n+\frac{1}{5}F_{n-1}-\frac{4}{5}\left(\frac{1}{2}\right)^n+\frac{4}{15}\left(\frac{1}{4}\right)^n+\frac{2}{15}\left(-\frac{1}{8}\right)^n\right]D_A+$$

$$\left[\frac{31}{20}-\frac{9}{10}F_n-\frac{1}{1+F_n}-\frac{1}{(1+F_n)^2}+\frac{7}{20}F_{n-1}-\right.$$

$$\left.\frac{F_n\,F_{n-1}}{(1+F_n)^2}+\frac{1-F_n}{1+F_n}\left(\frac{1}{2}\right)^n-\frac{1}{5}\left(\frac{1}{4}\right)^n\right]D'_A+$$

$$\left[-\frac{39}{20}+\frac{21}{10}F_n-(F_n)^2+\frac{1}{1+F_n}+\frac{1}{(1+F_n)^2}-\frac{3}{20}F_{n-1}+\right.$$

$$\left.\frac{F_n\,F_{n-1}}{(1+F_n)^2}+\frac{9}{5}\left(\frac{1}{2}\right)^n-\frac{2}{1+F_n}\left(\frac{1}{2}\right)^n+\frac{2}{15}\left(\frac{1}{4}\right)^n-\frac{2}{15}\left(-\frac{1}{8}\right)^n\right]D''_A,$$

where $D_A=\sum_{i=1}^{m}\sum_{j=1}^{m}p_i\,p_j\,(a_{ij})^2$, and as before (p. 137)

$$D'_A=\sum_{i=1}^{m}p_i\,(a_{ii})^2 \text{ and}$$

$$D''_A=\left(\sum_{i=1}^{m}p_i\,a_{ii}\right)^2.$$

The dominance covariance in generation n is thus a function of the latent roots and three genetic quantities which involve the gene frequencies and the dominance deviations of the original randomly mated population. These three quantities appear also in the dominance variance in generation n (LAGERVALL, 1961).

In inbred populations there is, unfortunately, a covariance of the genic value of one relative and the dominance deviation of another relative. This covariance is for full sibs in generation n of brother-sister mating (locus A)

(5 a) $\mathrm{Cov}_{G,D:A}$(F. S. gen. n) =

$$\left[-\frac{1}{2}+2\,F_n+\frac{1}{2}F_{n-1}+\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m}p_i\,\alpha_i^{(n)}\,a_{ii}^{(n)}+$$

$$2\left[1-F_n-\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m}p_i\,\alpha_i^{(n)}\sum_{j=1}^{m}p_j\,a_{ij}^{(n)}.$$

This covariance can also be written as

(5 b) $\mathrm{Cov}_{G,D:A}(\text{F. S. gen. n}) =$

$$\frac{F_n}{(1+F_n)^2}\left[-\frac{1}{2}(1-F_n)(1-F_{n-1})+(1+F_n)\left(\frac{1}{2}\right)^n\right]\times$$

$$(D'_A-D''_A)+\frac{1}{1+F_n}\left[-\frac{1}{2}(1-F_n)(1-F_{n-1})+(1+F_n)\left(\frac{1}{2}\right)^n\right]C_A,$$

provided there is no epistasis (or epistasis of only the type genic × genic) present. (D'_A, D''_A, and C_A are given on p. 137.)

Finally, the three fractions of the genetic covariance of full sibs can be added when no epistasis is present. The genetic covariance of full sibs in generation n is then, expressed in terms of the genetic parameters of the random-mating population, (locus A) =

(6) $\mathrm{Cov}_{H:A}(\text{F. S. gen. n}) =$

$$(1+2F_n+F_{n-1})G_A+\left[\frac{3}{5}-\frac{4}{5}F_n+\frac{1}{5}F_{n-1}-\right.$$

$$\left.\frac{4}{5}\left(\frac{1}{2}\right)^n+\frac{4}{15}\left(\frac{1}{4}\right)^n+\frac{2}{15}\left(-\frac{1}{8}\right)^n\right]D_A+\left[-\frac{9}{20}+\right.$$

$$\left.\frac{11}{10}F_n+\frac{7}{20}F_{n-1}+\left(\frac{1}{2}\right)^n-\frac{1}{5}\left(\frac{1}{4}\right)^n\right]D'_A+\left[\frac{1}{20}-\right.$$

$$\left.(F_n)^2+\frac{1}{10}F_n-\frac{3}{20}F_{n-1}-\frac{1}{5}\left(\frac{1}{2}\right)^n+\frac{2}{15}\left(\frac{1}{4}\right)^n-\right.$$

$$\left.\frac{2}{15}\left(-\frac{1}{8}\right)^n\right]D''_A+\left[-1+4F_n+F_{n-1}+2\left(\frac{1}{2}\right)^n\right]C_A.$$

This discussion has been limited to one locus. However, if several loci are involved and the different loci are independent, the total covariance of full sibs is obtained from the formulas given above by summation over loci. This independence of loci can be assumed to exist in a population that has been randomly mated for quite some time. Linked loci require, of course, a longer time to reach the point of random combination than unlinked loci.

Inclusion of epistasis of the type genic×genic probably would increase the total genetic covariance by one term, a term involving the genic×genic epistatic variance of the random-mating population. However, this has not been mathematically confirmed and has therefore to remain as a conjecture.

III. THE COVARIANCE OF PARENT AND OFFSPRING UNDER FULL-SIB MATING

The covariance of a parent in generation n—1 and an offspring in generation n can be treated in the same way as the covariance of full sibs in generation n. The genic covariance of parent and offspring obtained in this way (locus A)

(7 a) $\mathrm{Cov}_{G:A}(\text{P. gen. } n-1, \text{ O. gen. } n) =$

$$\left(4-\frac{6}{5}S_n-\frac{16}{5}S_{n+1}\right)\sum_{i=1}^{m} p_i\,\alpha_i^{(n-1)}\,\alpha_i^{(n)}$$

or

(7 b) $\mathrm{Cov}_{G:A}(\text{P. gen. } n-1, \text{ O. gen. } n) =$

$$(1+2\,F_n+F_{n-1})\sum_{i=1}^{m} p_i\,\alpha_i^{(n-1)}\,\alpha_i^{(n)}.$$

If this expression is divided by the geometric mean of the genic variances in generations n−1 and n, the correlation of the genic value of a parent and the genic value of an offspring is obtained. The genic correlation of a parent in generation n−1 and an offspring in generation n is thus

(8) $r^G_{\text{P. gen. } n-1,\ \text{O. gen. } n} =$

$$\frac{1+2\,F_n+F_{n-1}}{2\sqrt{(1+F_n)\,(1+F_{n-1})}}\times$$

$$\frac{\sum_{i=1}^{m} p_i\,\alpha_i^{(n-1)}\,\alpha_i^{(n)}}{\sqrt{\sum_{i=1}^{m} p_i\left[\alpha_i^{(n-1)}\right]^2\sum_{j=1}^{m} p_j\left[\alpha_j^{(n)}\right]^2}}.$$

The first part of this expression equals the coefficient of relationship between parent and offspring (WRIGHT, 1921, 1922). The second part equals 1, if $\alpha_i^{(n-1)}=\alpha_i^{(n)}$, *i.e.* under the following conditions:

1. The parent belongs to generation 0 and the offspring to generation 1 (random mating) or parent and offspring belong to generations with inbreeding coefficients of 1.
2. No dominance and epistasis (except epistasis of the type genic × genic) present.
3. No epistasis (except epistasis of the type genic × genic) present and only two alleles with gene frequencies 0.5 segregating at each locus.

In cases 2. and 3. $\alpha_i^{(n-1)} = \alpha_i^{(n)} = \alpha_i$.

The genic covariance of parent and offspring can also be expressed as a function of the genetic parameters of the original random-mating population. If no epistasis (or epistasis of the type genic × genic) is present (locus A)

(7 c) $\mathrm{Cov}_{G:A}$(P. gen. n−1, O. gen. n) =

$$(1+2\,F_n+F_{n-1})\,G_A+$$

$$\frac{F_n\,F_{n-1}\,(1+2\,F_n+F_{n-1})}{(1+F_n)\,(1+F_{n-1})}\,(D'_A-D''_A)+$$

$$\frac{(1+2\,F_n+F_{n-1})\,(F_n+F_{n-1}+2\,F_n\,F_{n-1})}{(1+F_n)\,(1+F_{n-1})}\,C_A.$$

The dominance covariance of parent in generation n−1 and offspring in generation n (locus A)

(9 a) $\mathrm{Cov}_{D:A}$(P. gen. n−1, O. gen. n) =

$$\left[-\frac{1}{2}+F_n+\frac{1}{2}F_{n-1}+\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m} p_i\,a_{ii}^{(n-1)}\,a_{ii}^{(n)}+$$

$$\left[\frac{1}{2}-F_n+\frac{1}{2}F_{n-1}-\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m} p_i\,a_{ii}^{(n-1)}\times$$

$$\sum_{j=1}^{m} p_j\,a_{ij}^{(n)}+\left[\frac{1}{2}-\frac{1}{2}F_{n-1}-\left(\frac{1}{2}\right)^n\right]\times$$

$$\left[\sum_{i=1}^{m} p_i\,a_{ii}^{(n)}\sum_{j=1}^{m} p_j\,a_{ij}^{(n-1)}+\sum_{i=1}^{m}\sum_{j=1}^{m} p_i\,p_j\,a_{ij}^{(n-1)}\,a_{ij}^{(n)}\right]+$$

$$2\left(\frac{1}{2}\right)^n\sum_{i=1}^{m}\sum_{j=1}^{m} p_i\,p_j\,a_{ij}^{(n-1)}\sum_{k=1}^{m} p_k\,a_{ik}^{(n)}.$$

Surprisingly enough, the latent roots $\frac{1}{4}$ and $-\frac{1}{8}$ are not included in this covariance while the root $\frac{1}{2}$ is.

It is obvious that in the original randomly mated population (n=1) the dominance covariance of parent and offspring equals 0. The dominance correlation between parent and offspring thus equals 0. So does also the dominance coefficient of relationship between parent and offspring (LAGERVALL, 1960).

In terms of the genetic parameters of the original randomly mated population, when no epistasis (or epistasis of only the type genic × genic) is present, the dominance covariance of parent and offspring (as regards locus A)

(9 b) $\mathrm{Cov}_{D:A}$(P. gen. n−1, O. gen. n.) =

$$\left[\frac{1}{2}-\frac{1}{2}F_{n-1}-\left(\frac{1}{2}\right)^n\right]D_A+\frac{1}{(1+F_n)(1+F_{n-1})}\times$$

$$\left[-\frac{1}{2}+F_n-\frac{1}{2}F_n F_{n-1}-\frac{1}{2}F_n (F_{n-1})^2+\right.$$

$$\left.\frac{1}{2}F_{n-1}+\left(\frac{1}{2}\right)^n-F_n F_{n-1}\left(\frac{1}{2}\right)^n\right]D'_A+$$

$$\frac{1}{(1+F_n)(1+F_{n-1})}\left[-\frac{1}{2}F_n+(F_n)^2+\right.$$

$$\frac{1}{2}F_n F_{n-1}-(F_n)^2 (F_{n-1})^2-\frac{1}{2}F_{n-1}+\frac{1}{2}(F_{n-1})^2+$$

$$\left.(F_n+2F_n F_{n-1}+F_{n-1})\left(\frac{1}{2}\right)^n\right]D''_A.$$

The covariance of the genic value of a parent in generation n−1 and the dominance deviation of an offspring in generation n (locus A)

(10 a) $\mathrm{Cov}_{G,D:A}$(P. gen. n−1, O. gen. n) =

$$\left[-\frac{1}{2}+2F_n+\frac{1}{2}F_{n-1}+\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m}p_i\,\alpha_i^{(n-1)}\,a_{ii}^{(n)}+$$

$$\left[2-2F_n-2\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m}p_i\,\alpha_i^{(n-1)}\sum_{j=1}^{m}p_j\,a_{ij}^{(n)}.$$

This covariance can also be written as

(10 b) $\mathrm{Cov}_{G,D:A}(\text{P. gen. } n-1,\ \text{O. gen. } n) =$

$$\frac{F_{n-1}}{1+F_{n-1}}\left[-\frac{(1-F_n)\,(1-F_{n-1})}{2\,(1+F_n)}+\left(\frac{1}{2}\right)^n\right](D'_A-D''_A)+$$

$$\left[-\frac{(1-F_n)\,(1-F_{n-1})}{2\,(1+F_n)}+\left(\frac{1}{2}\right)^n\right]C_A,$$

when no epistasis (or epistasis of only the type genic×genic) is present.

The covariance of the dominance deviation of a parent in generation n−1 and the genic value of an offspring in generation n (locus A)

(11 a) $\mathrm{Cov}_{D,G:A}(\text{P. gen. } n-1,\ \text{O. gen. } n) =$

$$\left[-\frac{1}{2}+F_n+\frac{3}{2}F_{n-1}+\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m}p_i\,\alpha_i^{(n)}\,a_{ii}^{(n-1)}+$$

$$\left[2-2\,F_{n-1}-2\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m}p_i\,\alpha_i^{(n)}\sum_{j=1}^{m}p_j\,a_{ij}^{(n-1)}$$

or with the same restrictions as regards epistasis as before

(11 b) $\mathrm{Cov}_{D,G:A}(\text{P. gen. } n-1,\ \text{O. gen. } n) =$

$$\frac{F_n}{1+F_n}\left[-\frac{(1-2\,F_n+F_{n-1})\,(1-F_{n-1})}{2\,(1+F_{n-1})}+\left(\frac{1}{2}\right)^n\right](D'_A-D''_A)+$$

$$\left[-\frac{(1-2\,F_n+F_{n-1})\,(1-F_{n-1})}{2\,(1+F_{n-1})}+\left(\frac{1}{2}\right)^n\right]C_A.$$

The genetic covariance of a parent in generation n−1 and an offspring in generation n is now (locus A, no epistasis)

(12) $\mathrm{Cov}_{H:A}(\text{P. gen. } n-1,\ \text{O. gen. } n) =$

$$(1+2\,F_n+F_{n-1})\,G_A+$$

$$\left[\frac{1}{2}-\frac{1}{2}F_{n-1}-\left(\frac{1}{2}\right)^n\right]D_A+$$

$$\left[-\frac{1}{2}+F_n+\frac{1}{2}F_{n-1}+\left(\frac{1}{2}\right)^n\right]D'_A-$$

$$F_n F_{n-1} D''_A +$$

$$\left[-1 + 3 F_n + 2 F_{n-1} + 2\left(\frac{1}{2}\right)^n\right] C_A.$$

IV. DISCUSSION

It has here been shown that, in the case of no epistasis, the genetic covariance of full sibs in generation n of brother-sister mating or of a parent in generation n−1 and an offspring in generation n can be expressed in terms of five genetic quantities which are functions of the genetic parameters of the original randomly mated population and of the latent roots of the generation matrix. These five genetic quantities are identical with those five that were used to describe the changes of the genic variance and of the dominance variance with inbreeding (LAGERVALL, 1961), when epistasis was not present.

How the coefficients of these five genetic quantities change with the inbreeding is depicted in Fig. 1 for the full-sib covariance and in Fig. 2 for the parent-offspring covariance. In both cases autosomal and sex-linked inheritance are considered. The autosomal inheritance was dealt with above, while the sex-linked inheritance is treated in an Appendix. In the sex-linked case it is assumed that only the X-chromosome contains genes affecting the trait studied and the Y-chromosome is devoid of any such genes. This may not be quite true for quantitative characters (MATHER, 1949); in case the Y-chromosome contains the same genes as the X-chromosome, the homo- and heterogametic sexes behave in the same way, as the homogametic sex in Figs. 1 and 2.

In Fig. 1 the coefficients in the autosomal case and corresponding coefficients in the sex-linked case are in all instances (except when F=0 or F=1) different. However, the bias that will arise by treating the sex-linked genes as autosomal genes is negligible, unless the sex-linked genes play a large role in determining the character under study.

In Fig. 2 there is no difference between the coefficients of the autosomal case and those of the sex-linked case, in which parent and offspring are both of the homogametic sex.

If each litter (or full-sib family) is considered as an inbred line, the covariance of full sibs equals the variance between lines. The genetic variance between lines has been computed in this way for the first three examples given by LAGERVALL (1961). All these examples dealt with only one locus, Ex. 1 when the gene frequencies were 0.5 and 0.5, Ex. 2

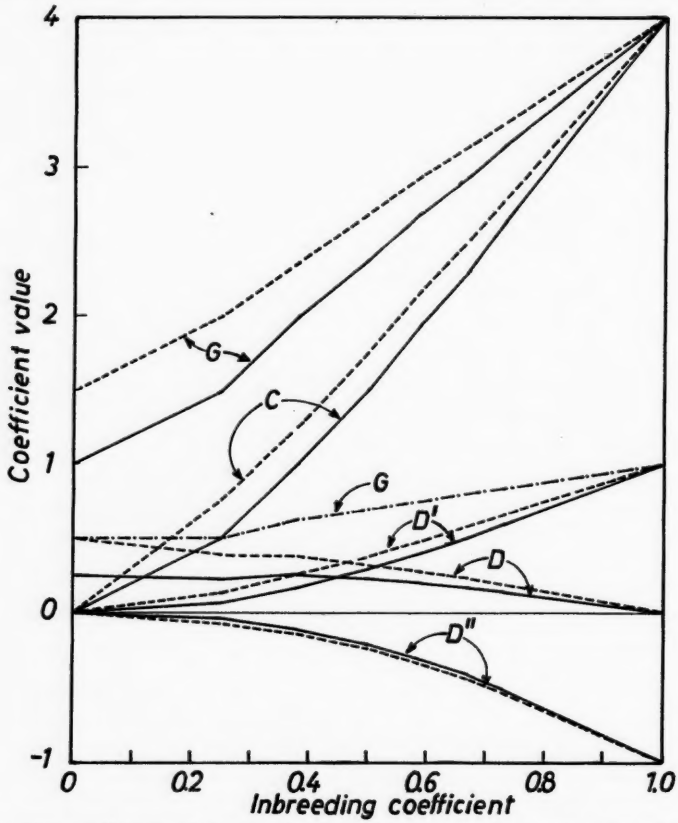

Fig. 1. The coefficients of the genetic quantities G, D, D′, D″, and C (see pp. 137 and 139) in the covariance of full sibs at different degrees of inbreeding under full-sib mating, when no epistasis is present.

——— autosomal inheritance,
– – – sex-linked inheritance, full sibs of homogametic sex,
—·—·— sex-linked inheritance, full sibs of heterogametic sex.

when the frequency of the plus-gene was 0.7 and that of the minus-gene 0.3, Ex. 3 when the frequency of the plus-gene was 0.9 and that of the minus-gene 0.1. Five different degrees of dominance were treated in each example. The genetic variance between lines for Ex. 1 is shown in Fig. 3, for Ex. 2 in Fig. 5 and for Ex. 3 in Fig. 7. Corresponding genetic variances within lines are shown in Figs. 4, 6, and 8, respectively.

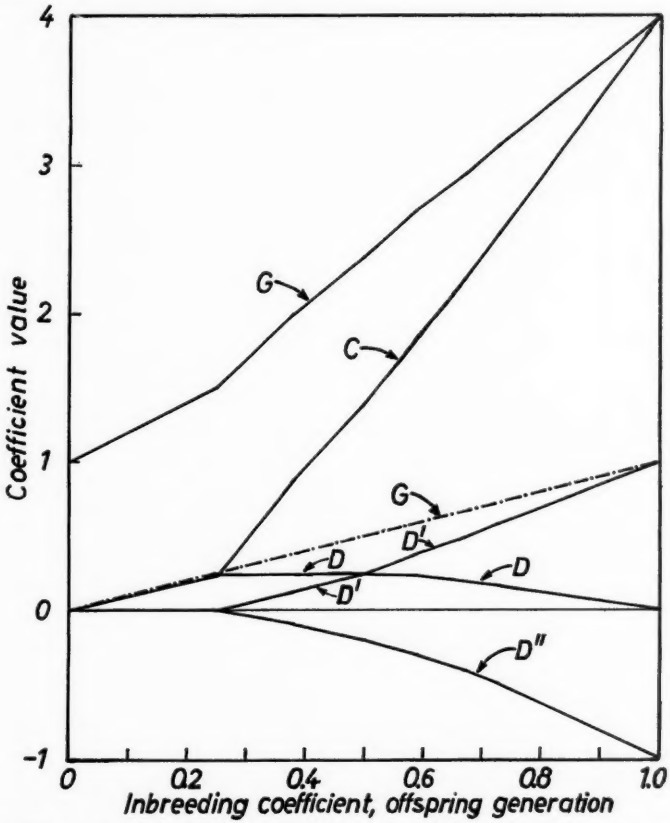

Fig. 2. The coefficients of the genetic quantities G, D, D′, D″, and C (see pp. 137 and 139) in the covariance of parent and offspring at different degrees of inbreeding under full-sib mating, when no epistasis is present.

——— autosomal inheritance and sex-linked inheritance, parent and offspring of homogametic sex,

—·—·— sex-linked inheritance, parent and offspring of heterogametic sex.

In Ex. 1 the genetic variance between lines increases almost linearly with the inbreeding, when dominance is lacking, partial or complete (Fig. 3). With overdominance there is an increase up to a maximum value and thereafter a decrease. The location of the maximum depends upon the degree of overdominance.

In Ex. 2 a maximum point is shown by the variance between litters only for the highest degree of overdominance, while the variance for

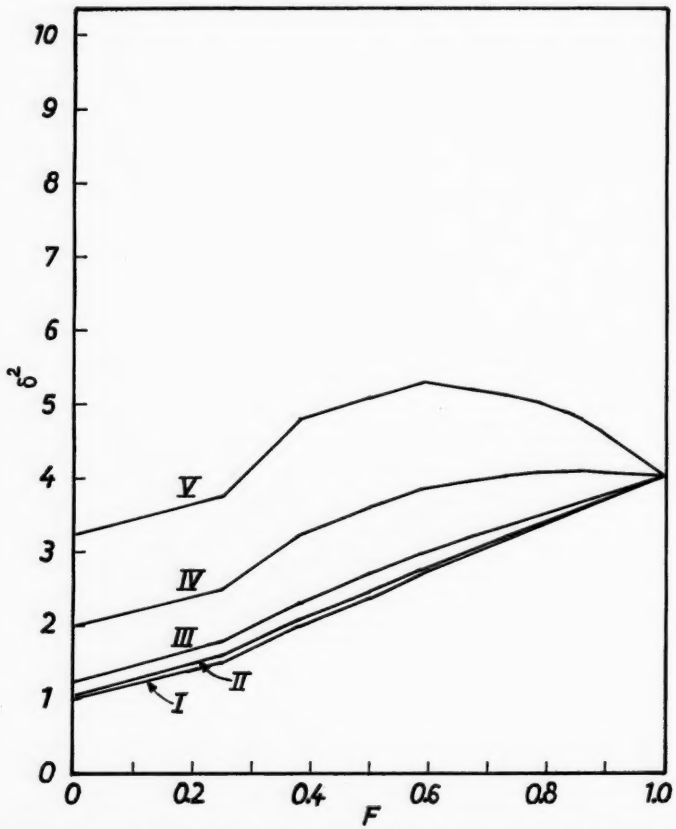

Fig. 3. The variance between lines (covariance of full sibs), σ^2, under full-sib mating at different degrees of inbreeding, F, and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, two alleles, gene frequencies 0.5. For further details, see text!

the lowest degree of overdominance increases in a linear fashion (Fig. 5), as is also the case, when dominance is lacking, partial or complete.

In Ex. 3 no maximum point occurs but a minimum point when overdominance is present (Fig. 7). In the cases where overdominance is not present, the increase is still almost a straight line. The case with complete dominance has already been dealt with by ROBERTSON (1952).

The variance within lines shows in Ex. 1 (Fig. 4) a steady decline to 0 for all degrees of dominance, also for the case of no dominance.

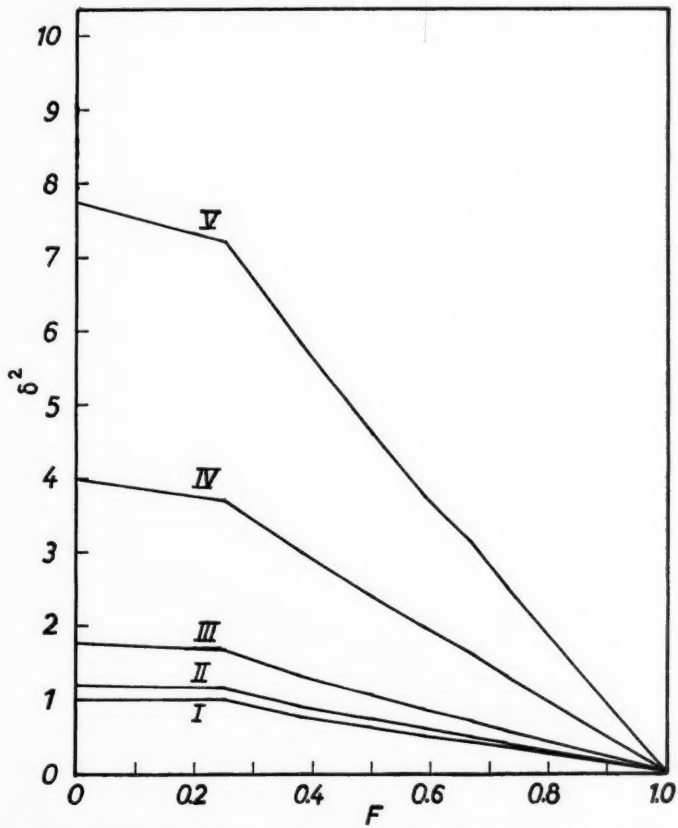

Fig. 4. The variance within lines, σ^2, under full-sib mating at different degrees of inbreeding, F, and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, two alleles, gene frequencies 0.5. For further details, see text!

In Ex. 2 (Fig. 6) a maximum is shown, at F=0.25, when dominance is present; with no dominance present the variance within lines decreases steadily as in the previous example. Ex. 3 (Fig. 8) has a pattern similar to that in Ex. 2.

In all examples the genetic variance within lines is increased considerably by overdominance at all inbreeding levels, except when F=1. When F=1, no genetic variance exists within lines. The effect of over-

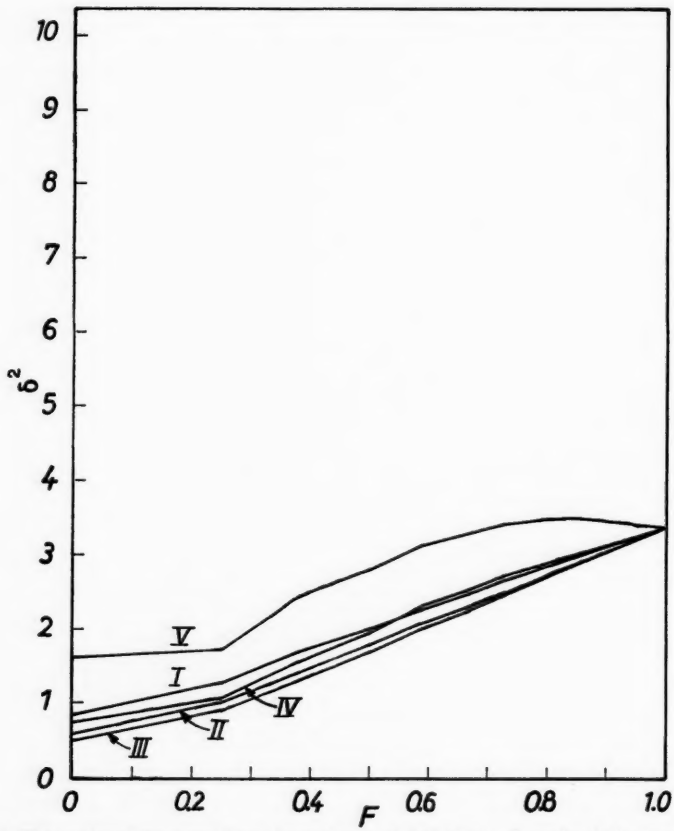

Fig. 5. The variance between lines (covariance of full sibs), σ^2, under full-sib mating at different degrees of inbreeding, F, and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, two alleles, gene frequencies 0.3 and 0.7. For further details, see text!

dominance on the genetic variance between lines is not as conspicuous as on the genetic variance within lines.

As to the possibilities of estimating the five genetic quantities mentioned above from the covariances of relatives in inbred populations, this has to be judged from case to case. The most important factors to take into consideration, when species and character are to be chosen, are if it is possible or not to estimate the environmental part of the covariance of relatives of the kind to be studied, and if epistasis is im-

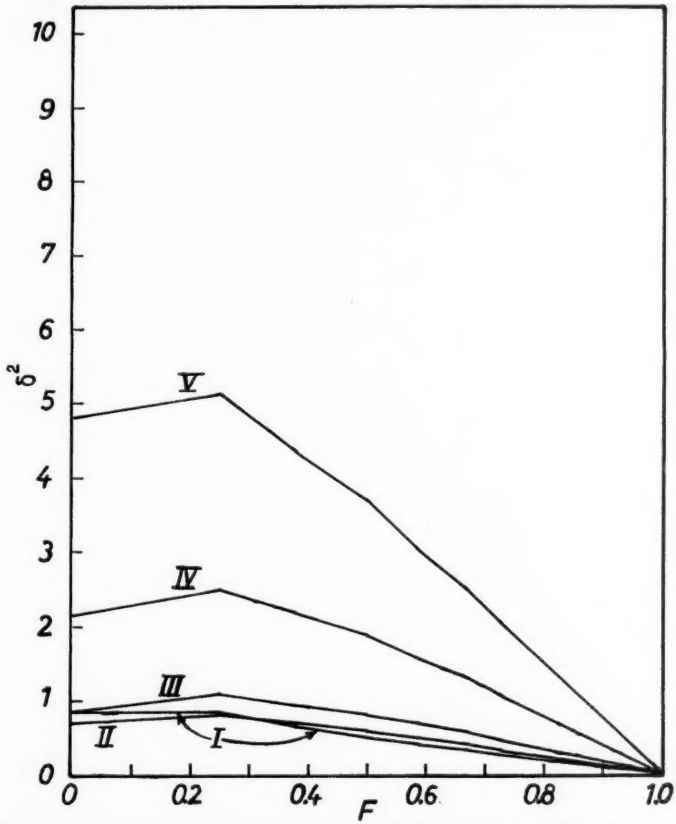

Fig. 6. The variance within lines, σ^2, under full-sib mating at different degrees of inbreeding, F, and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, two alleles, gene frequencies 0.3 and 0.7. For further details, see text!

portant or not in the inheritance of the character to be investigated. Any general answers to these two questions cannot be given.

It is likely that if genic×genic epistasis were included this would increase the number of unknown genetic quantities by only one. However, this has not been confirmed and must therefore remain a conjecture at the present.

As the unknown genetic quantities are functions of covariances of biological variables which in general are normally distributed, standard

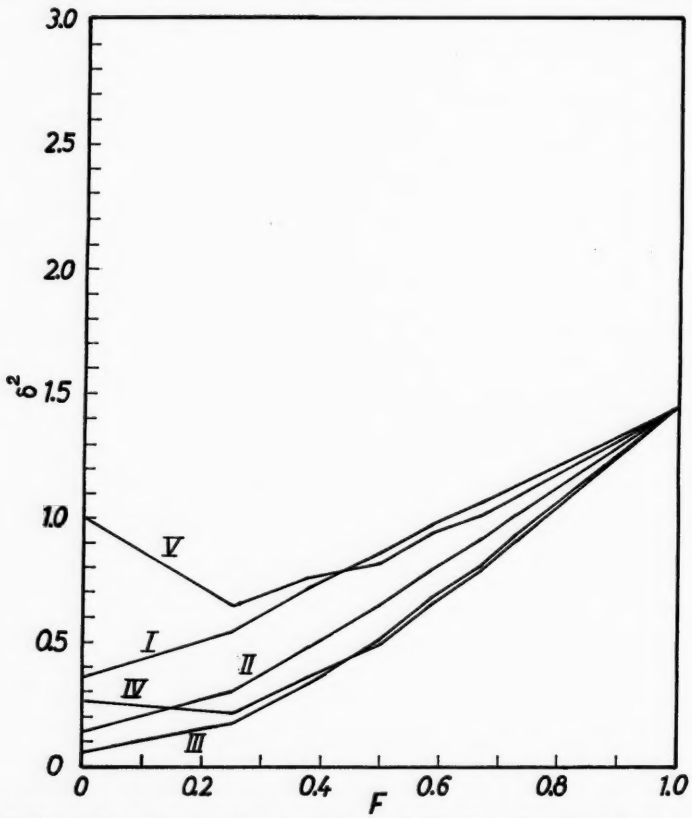

Fig. 7. The variance between lines (covariance of full sibs), σ^2, under full-sib mating at different degrees of inbreeding, F, and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, two alleles, gene frequencies 0.1 and 0.9. For further details, see text!

errors of the different genetic quantities can be obtained with the help of the moment generating or characteristic function of the multivariate normal distribution (see *e.g.* ANDERSON, 1958, or REEVE, 1955).

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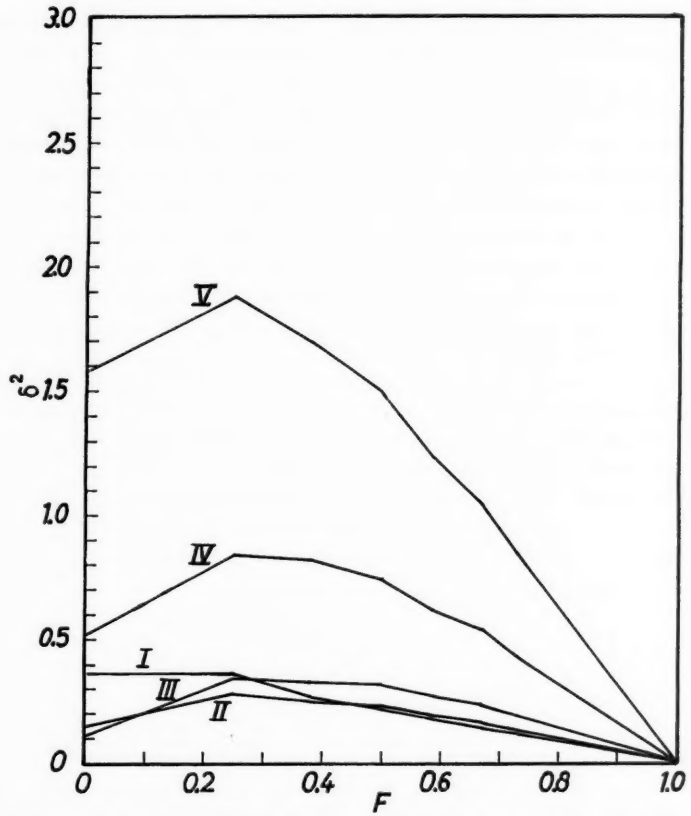

Fig. 8. The variance within lines, σ^2, under full-sib mating at different degrees of inbreeding, F, and dominance (I no dom.; II partial dom.; III complete dom.; IV overdom., case 1; V overdom., case 2). One locus, two alleles, gene frequencies 0.1 and 0.9. For further details, see text!

SUMMARY

The genetic covariances of full sibs and of parent and offspring under full-sib mating are investigated. For simplicity, it is assumed that alleles at different loci are randomly combined and further that no epistasis is present. The general case with any number of loci and any number of alleles at each locus is treated.

Fractions of the genetic covariance due to genic effects, dominance, and genic effects in one relative and dominance in the other as well as

the total genetic covariance are developed. It is shown that the genic correlation equals the coefficient of relationship, if the two relatives belong to the same generation or to generations with inbreeding coefficients of either 0 or 1, or if no dominance is present, or if dominance is present and only two alleles with gene frequencies 0.5 are segregating. The dominance correlation equals the dominance coefficient of relationship under random mating, in inbred populations this correlation has a very complex structure. The total genetic covariance is found to be a function of the latent roots of the generation matrix and the same five genetic quantities that described the changes of the genic variance and the dominance variance under inbreeding, when the same restrictions as in this study apply.

A comparison is made of the coefficients of the five genetic quantities for autosomal genes and for sex-linked genes. A bias is introduced in most instances by treating the sex-linked genes as autosomal genes, but this bias is probably negligible in most cases.

The genetic variances between lines (covariance of full sibs) and within lines at different inbreeding levels are estimated in a few examples of one-locus cases with two alleles of different gene frequencies and with different degrees of dominance.

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Appendix

The Covariance of Full Sibs and of Parent and Offspring at Full-Sib Mating for Sex-Linked Genes.

For convenience, males are regarded as the heterogametic sex and females as the homogametic sex. Furthermore, the Y-chromosome is considered to lack genes affecting the trait under investigation. Four different mating types can then be distinguished in the full-sib mating system (males first):

1. a×aa with frequency r
2. a×ab „ „ s
3. a×bb „ „ t
4. a×bc „ „ u

The generation equation then becomes

$$\begin{pmatrix} r^{(n)} \\ s^{(n)} \\ t^{(n)} \\ u^{(n)} \end{pmatrix} = \begin{pmatrix} 1 & \frac{1}{4} & 0 & 0 \\ 0 & \frac{1}{2} & 1 & \frac{1}{2} \\ 0 & \frac{1}{4} & 0 & 0 \\ 0 & 0 & 0 & \frac{1}{2} \end{pmatrix} \begin{pmatrix} r^{(n-1)} \\ s^{(n-1)} \\ t^{(n-1)} \\ u^{(n-1)} \end{pmatrix}$$

where $r^{(n)}$ = the frequency of the mating type a×aa in generation n of full-sib mating, etc.

When $r^{(0)} = s^{(0)} = t^{(0)} = 0$ and $u^{(0)} = 1$ (*i.e.* the inbreeding is started with unrelated, noninbred individuals), diagonalization of this generation matrix gives

$$r^{(n)} = 1 - \frac{2}{5} S_n - \frac{7}{5} S_{n+1} + \frac{1}{2}\left(\frac{1}{2}\right)^n$$

$$s^{(n)} = \frac{1}{5} S_n + \frac{6}{5} S_{n+1} - \left(\frac{1}{2}\right)^n$$

$$t^{(n)} = \frac{1}{5} S_n + \frac{1}{5} S_{n+1} - \frac{1}{2}\left(\frac{1}{2}\right)^n$$

$$u^{(n)} = \left(\frac{1}{2}\right)^n,$$

where, as before, $S_n = \varepsilon^n + (\varepsilon')^n$, etc.

Let there be m alleles at the locus studied, the B-locus, in the original randomly mated population, B_1, B_2, ..., B_m. Let the gene frequencies (at genetic equilibrium) be q_1, q_2, ..., q_m, respectively. Then the following frequencies of the following kinds of matings are obtained in generation n:

Kind of mating	*Frequency*
$B_i \times B_i B_i$	$q_i\, r^{(n)} + (q_i)^2\, s^{(n)} + (q_i)^2\, t^{(n)} + (q_i)^3\, u^{(n)}$
$B_i \times B_i B_j$	$q_i\, q_j\, s^{(n)} + 2\,(q_i)^2\, q_j\, u^{(n)}$
$B_i \times B_j B_j$	$q_i\, q_j\, t^{(n)} + q_i\,(q_j)^2\, u^{(n)}$
$B_i \times B_j B_k$	$2\, q_i\, q_j\, q_k\, u^{(n)}$

or

Kind of mating	*Frequency*
$B_i \times B_i B_i$	$q_i - \frac{2}{5} q_i\,(1-q_i)\, S_n - \frac{7}{5} q_i\,(1-q_i)\, S_{n+} \div \frac{1}{2} q_i\,(1-q_i)\,(1-2\,q_i)\left(\frac{1}{2}\right)^n$
$B_i \times B_i B_j$	$\frac{1}{5} q_i\, q_j\, S_n + \frac{6}{5} q_i\, q_j\, S_{n+1} - q_i\, q_j\,(1-2\,q_i)\left(\frac{1}{2}\right)^n$

$$B_i \times B_j B_j \quad \frac{1}{5} q_i q_j S_n + \frac{1}{5} q_i q_j S_{n+1} - \frac{1}{2} q_i q_j (1 - 2 q_j) \left(\frac{1}{2}\right)^n$$

$$B_i \times B_j B_k \quad 2 q_i q_j q_k \left(\frac{1}{2}\right)^n.$$

The following genetic model is adopted for the males:

$$y_{B_i}^{(n)} = \beta_i^{(n)} + \mu^{(n)},$$

where $y_{B_i}^{(n)}$ = the genetic value of the "genotype" B_i in generation n of full sibbing,
$\beta_i^{(n)}$ = the effect of the sex-linked gene B_i in generation n,
$\mu^{(n)}$ = the mean genetic value of all males in generation n.

But $\beta_i^{(n)} = \beta_i$, the effect of the gene B_i under random mating. It can be shown that the genic (here also = genetic) covariance of full brothers in generation n as regards locus B

(App. 1) $\mathrm{Cov}_{G:B}$(F. Broth. gen. n) =

$$\left(\frac{1}{2} + \frac{1}{2} F_{n-1}\right) \sum_{i=1}^{m} q_i (\beta_i)^2,$$

and that the genic (= genetic) covariance of sire in generation n − 1 and son in generation n

(App. 2) $\mathrm{Cov}_{G:B}$(Sire gen. n − 1, Son gen. n) =

$$F_n \sum_{i=1}^{m} q_i (\beta_i)^2,$$

where the inbreeding coefficients are used in the same sense as before (autosomal inheritance). As regards sex-linked inheritance the inbreeding coefficient does not have any significance in the heterogametic sex.

For females the following genetic model is adopted:

$$y_{B_iB_j}^{(n)} = \beta_i^{(n)} + \beta_j^{(n)} + b_{ij}^{(n)} + \mu^{(n)},$$

where $y_{B_iB_j}^{(n)}$ = the genetic value of the genotype B_iB_j in generation n of brother-sister mating,
$\beta_i^{(n)}$ = the effect of the sex-linked gene B_i in generation n (not necessarily = $\beta^{(n)}$ in the males),
$b_{ij}^{(n)}$ = the dominance deviation of the genotype B_iB_j in generation n,

$\mu^{(n)}$ = the mean genetic value of all females in generation n (not necessarily = $\mu^{(n)}$ in the males).

The genic covariance of full sisters in generation n is now as regards locus B:

(App. 3) $\mathrm{Cov}_{G:B}(\text{F. Sist. gen. n}) =$

$$\left(\frac{3}{2} + 2\,F_n + \frac{1}{2}F_{n-1}\right)\sum_{i=1}^{m} q_i \left[\beta_i^{(n)}\right]^2$$

(the genic covariance of full sisters in a random-mating population is thus $\frac{3}{4}$ of the genic sex-linked variance for females and not $\frac{1}{2}$ as KEMPTHORNE (1957) stated; this can, however, be shown in much simpler ways). In this case the inbreeding coefficients at sex-linked inheritance and those at autosomal inheritance are identical.

The dominance covariance of full sisters in generation n

(App. 4) $\mathrm{Cov}_{D:B}(\text{F. Sist. gen. n}) =$

$$\left[\frac{3}{4} - F_n + \frac{1}{4}F_{n-1} - \frac{1}{2}\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m}\sum_{j=1}^{m} q_i\, q_j \left[b_{ij}^{(n)}\right]^2 +$$

$$\left[-\frac{1}{4} + F_n + \frac{1}{4}F_{n-1} + \frac{1}{2}\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m} q_i \left[b_{ii}^{(n)}\right]^2 +$$

$$\left[\frac{1}{2} - \frac{1}{2}F_{n-1} - \left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m} q_i\, b_{ii}^{(n)} \sum_{j=1}^{m} q_j\, b_{ij}^{(n)} +$$

$$\left(\frac{1}{2}\right)^n \sum_{i=1}^{m} q_i \left[\sum_{j=1}^{m} q_j\, b_{ij}^{(n)}\right]^2$$

(the dominance covariance of full sisters under random mating thus equals $\frac{1}{2}$ of the sex-linked dominance variance for females and not $\frac{1}{4}$ as KEMPTHORNE (1957) stated; also this can be shown in much simpler ways).

The covariance of the genic value of one full sister and the dominance deviation of another full sister in generation n (locus B)

(App. 5) $\mathrm{Cov}_{G,D:B}(\text{F. Sist. gen. n}) =$

$$\left[-\frac{1}{4}+2\,F_n+\frac{1}{4}F_{n-1}+\frac{1}{2}\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m} q_i\,\beta_i^{(n)}\,b_{ii}^{(n)}+$$

$$\left[2-2\,F_n-\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m} q_i\,\beta_i^{(n)}\sum_{j=1}^{m} q_j\,b_{ij}^{(n)}.$$

The relations between the $\beta_i^{(n)}$'s and $b_{ij}^{(n)}$'s on the one hand and the β_i's and b_{ij}'s on the other hand are for sex-linked genes in females the same as for autosomal genes. The sex-linked genetic covariance of full sisters in generation n can therefore, when no epistasis is present, be written as

(App. 6) $\mathrm{Cov}_{H:B}(\text{F. Sist. gen. n}) =$

$$\left(\frac{3}{2}+2\,F_n+\frac{1}{2}F_{n-1}\right)\sum_{i=1}^{m} q_i\,(\beta_i)^2+$$

$$\left[\frac{3}{4}-F_n+\frac{1}{4}F_{n-1}-\frac{1}{2}\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m}\sum_{j=1}^{m} q_i\,q_j\,(b_{ij})^2+$$

$$\left[-\frac{1}{4}+F_n+\frac{1}{4}F_{n-1}+\frac{1}{2}\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m} q_i\,(b_{ii})^2-(F_n)^2\left(\sum_{i=1}^{m} q_i\,b_{ii}\right)^2+$$

$$\left[-\frac{1}{2}+4\,F_n+\frac{1}{2}F_{n-1}+\left(\frac{1}{2}\right)^n\right]\sum_{i=1}^{m} q_i\,\beta_i\,b_{ii}.$$

The covariances of dam in generation n−1 and daughter in generation n are in all cases identical with corresponding parent-offspring covariances at autosomal inheritance. These covariances are therefore not repeated here; reference is made to formulas 7 b and c, 9 a and b, 10 a and b, 11 a and b, and 12.

BRIEF REPORT

SUSHIL K. BOSE, GÖSTA ZETTERBERG and NILS FRIES: Reactivation of *Ophiostoma* cells photodynamically inactivated with visible light.

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In connection with some experiments designed to induce mutations in the fungus *Ophiostoma* by means of visible white light it appeared that the post-illumination conditions greatly influenced the number of survivors. An analysis of the results showed that the number of conidia surviving increased when the time between the end of the illumination period and the plating out of the conidia in the nutrient agar medium was increased. During this period the conidia remained in aqueous solution in the dark. Whereas effects of this sort are well-known in cells inactivated by ultraviolet light with (GEISSLER, 1959) or without (HOLLAENDER and CLAUS, 1936; ROBERT and ALDOUS, 1949) an added photoreceptor, no reactivation after treatment with visible light appears to have been observed earlier (ZELLE and HOLLAENDER, 1956; JAGGER, 1958). Therefore it seemed necessary to perform some experiments in order to establish the reality of the phenomenon.

The living material consisted of the uninucleate conidia of *Ophiostoma multiannulatum*, strain 51 ("star"), produced in continuously shaken flasks with 20 ml of liquid minimal medium N 6 (FRIES, 1949). With an inoculum of 10^8 conidia per flask the culture had just entered the stationary phase of growth after 24 hours. At that time the conidia, circa 10^{10} per flask, were centrifuged off, washed twice with distilled water, filtered through cotton-wool and resuspended in distilled water containing 0.005 per cent erythrosine. This suspension, diluted to a density of $5 \cdot 10^7$ conidia per ml was shaken mechanically in a dark room for $5^1/_2$ hours and then illuminated in a glass Büchner funnel with a sintered glass bottom through which oxygen was bubbled. The funnel was kept in a water bath at $+10°$ C. The source of illumination was a Philips, 24 volt, 150 W, lamp, placed at a distance of 7.5 cm from the glass funnel.

After 45 minutes' illumination a survival count of c. 2 per cent was obtained from platings made immediately after the illumination period. However, if the illuminated conidia were kept in the suspension for some time in darkness two to five times higher survival counts were obtained. This increase in the number of conidial survivors was studied in three experiments, the results of which are reproduced in Fig. 1.

The slower inactivation of the conidia in Expt. 3 (compared with that in the other two experiments) may have been due to inaccurate focussing of the lamp; the relatively slower increase in the number of conidial survivors in this experiment can not be explained as yet, however.

The reactivation process which occurs in the aqueous solution of erythrosine obviously comes to an end as soon as the cells are plated out in the nutrient agar medium. This could mean that the healing of the damage caused by the light requires a relatively slow metabolism. Preliminary experiments with reactivation in liquid nutrient solutions do not seem to support this interpreta-

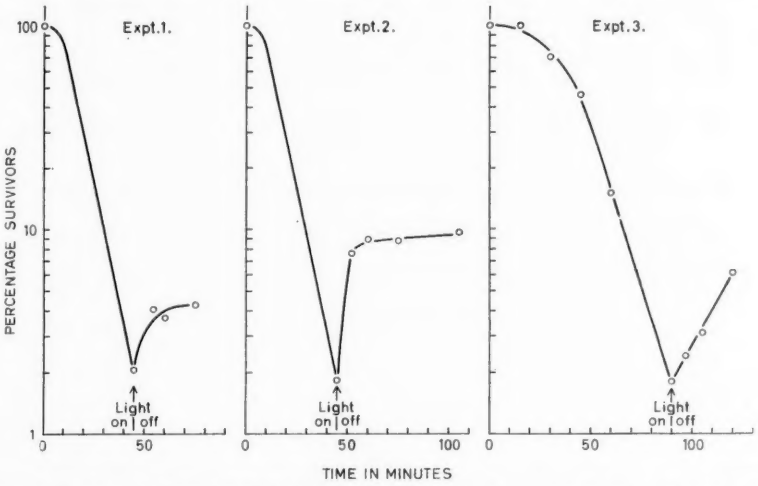

Fig. 1. The survival counts of *Ophiostoma* conidia in an aqueous solution of erythrosine after a period of illumination with visible light and during the following period in darkness.

tion. The experiments reported above do, however, show that it is also necessary to pay attention to the postillumination procedure when inactivating cells photodynamically with visible light.

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